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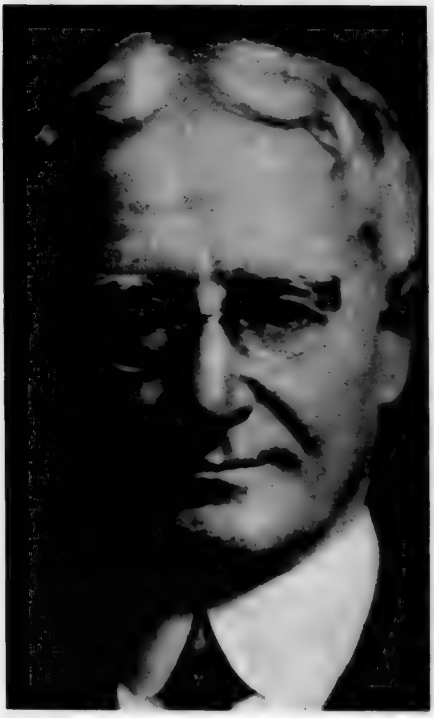

DR. WILLIAM J. GIES

William J. Gies, Founder and Editor-emeritus of the JOURNAL OF DENTAL RESEARCH, celebrates his seventieth birthday on February 21 of this year. To the readers of the JOURNAL it is entirely unnecessary to list Dr. Gies' accomplishments, his contributions to dentistry and to science as a whole, and his honors. On page one of Volume One of the JOURNAL, Dr. Gies stated that, "Research is the mainspring in the chronometer of science." Dr. Gies has been one of the most persistent "winder-uppers" of that mainspring for dental science. This JOURNAL and *The International Association for Dental Research* stand as records of achievement. Dr. Gies refused to grow old when retired from active teaching five years ago and we feel certain that attainment of three score and ten will merely be another mile post along the road of a full and useful life.

H. B. G. R.

THE NEW FORMAT OF THE JOURNAL OF DENTAL RESEARCH

Since the founding of the JOURNAL OF DENTAL RESEARCH in 1919 its pages have been printed in 11 on 13 #31 type with a type page 26 x 42 picas. This format has become familiar to the readers and a change will naturally result in comment. Over three months ago our printers suggested certain changes in many of their 73 periodicals. The use of the same format in most of these journals will permit simplifications that will save many hours and speed up production. In addition the use of the new format, 10 on 12 #8 type with a 29 x 47 picas type page, will conserve much essential material. The amount of paper saved by enlarging the type page and reducing the type size is apparent. The page size will not be changed so that bound volumes will be consistent in size. In doing its bit for the defense of our nation the JOURNAL will not only aid in conserving scarce materials but will be able to bring more written material to its readers in a given number of pages. The new format is introduced with this issue.

H. B. G. R.

CONSERVATION OF SCHOLARLY JOURNALS

The American Library Association created this last year the Committee on Aid to Libraries in War Areas, headed by John R. Russell, Librarian of the University of Rochester. The Committee is faced with numerous serious problems and hopes that American scholars and scientists will be of considerable aid in the solution of one of these problems.

One of the most difficult tasks in library reconstruction after the first World War was that of completing foreign institutional sets of American scholarly, scientific, and technical periodicals. The attempt to avoid a duplication of that situation is now the concern of the Committee.

Many sets of journals will be broken by the financial inability of the institutions to renew subscriptions, more will have been broken through mail difficulties and loss of shipments, while still other sets will have disappeared in the destruction of libraries. As far as possible they will be completed from a stock of periodicals being purchased by the Committee. The size of the eventual demand is impossible to estimate, but requests received by the Committee already give evidence that it will be enormous.

Fearing this possible reduction in the already limited supply of scholarly and scientific journals, the Committee hopes to enlist the cooperation of subscribers to this journal in preventing the sacrifice of this type of material to the demands for old periodicals for pulp. It is scarcely necessary to mention the appreciation of foreign institutions and scholars for this activity.

Questions concerning the project or concerning the value of particular periodicals to the project should be directed to Wayne M. Hartwell, Executive Assistant to the Committee on Aid to Libraries in War Areas, Rush Rhees Library, University of Rochester, Rochester, New York.

POSTEXTRACTION USE OF SULFANILAMIDE, LOCALLY[1]

FERDINAND GRIESS, D.D.S.

University of Nebraska, Lincoln, Nebr.

Practitioners of dentistry should realize that, although sulfanilamide is a drug possessing extraordinary therapeutic powers, it may at the same time produce a wide range of toxic manifestations. Some of these are mild and of no importance while others may be of such severity as to endanger the life of the patient. The dosage used in the local treatment of surgical wounds is so small, however, that the possibility of injurious toxicity resulting from local treatment seems rather remote. Local implantation of sulfanilamide appears to be a highly efficient method of preventing and combating infection. Systemic administration of the drug aims at producing a concentration of from 10 to 16 mg. per 100 cc. in the blood and tissue fluids. Local implantation produces a concentration of about 800 mg. per 100 cc. in the area affected, and maintains this concentration over a period of about 36 hours. In view of the facts stated above, the local use of pulverized sulfanilamide as a preventive measure against infections should be given serious consideration.

At the University of Nebraska, we have carried on an experiment for the past two years under the same conditions in all cases. Two graduate nurses are in attendance at all times during operations in the surgical operating room. The operator and assistant work under conditions which are as sterile as are those which obtain for any major operation, and a floating nurse is always in attendance. All packs, instruments, linens, and appliances are absolutely sterile.

In our experimentation, we maintained a control group without medication of any kind. This control group numbered 129 operations with a total of 291 teeth extracted (122 maxillary and 169 mandibular). Of this group, 115 were operated upon under local anesthesia, and 14 under general anesthesia; 14 were mandibular impactions; 8 were maxillary impactions; 14 were alveolectomies, and one was a supernumerary tooth. In the control group, there were 2 cases of cellulitis, both occurring in impacted and partially erupted mandibular third molars. There were also 8 cases of dry sockets (7 mandibular and 1 maxillary).

With a group treated by the local use of pulverized sulfanilamide, we expected to make a report on approximately the same number of operations as constituted the control group. The success of this latter treatment, however, was so startling that the procedure was continued, and we now have records on 731 operations with a total number of 1672 teeth extracted (699 maxillary and 760 mandibular). Of these, 606 were under local anesthesia, and 125 under general

[1] Received for publication September 15, 1941.

anesthesia; 64 were mandibular impactions, some partial and some complete: 42 were maxillary impactions; 57 were alveolectomies; and 16 were supernumerary teeth.

In this experimental group, there was one case of cellulitis, which occurred in a child 5 years of age upon the removal of a mandibular deciduous first molar under general anesthesia (Vinethene); there was also one dry socket occurring in the case of a mandibular first molar in which the pulp had died. In all cases of apicoectomies, residual infections, and radicular cysts, the cavities were filled with pulverized sulfanilamide and closed immediately. To date, all of these cases have been successful, with very little pain and with less swelling than appeared in the control series. In comparison with the results in the control series, our experience under this method with partially or completely unerupted third molars was most impressive. Cases treated with sulfanilamide showed a noticeable decrease in constitutional reactions: pain, swelling, trismus, and lymphadenitis.

There was no noticeable difference in the two series with respect to postoperative hemorrhage. The amount of pulverized sulfanilamide used was from 5 gr. to 30 gr., packed into the cavities without washing or drying out the cavity. In all alveolectomies and impactions, the tissues were sutured. In the simple extractions, a gauze pack filled with the pulverized sulfanilamide was placed over the cavity, and the patient told to bite on this for the following 15 minutes. At no time was there any evidence of toxic reaction from the drug.

COMMENTS

The effects of local sulfanilamide therapy are reduction of postoperative pain, noticeable reduction in the rise of temperature, and marked control of reactionary trismus and swelling.

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ROOT CANAL STUDIES: ANAEROBIC CULTURES[1]

FRED W. MORSE, JR., M.D. AND MORTON F. YATES, D.M.D.

Harvard University Dental School, Boston, Mass.

In studies of the bacteriology of root canals we find a considerable number of negative cultures obtained on the initial opening of the root canal. Since we are supposedly dealing with an infected pulp and canal, it seems to us that this may well cause concern. The question, therefore, arises: Are we using the best methods to obtain complete bacteriological information on root canals undergoing treatment.

Sommer (1) has mentioned that he gets a considerable number of negative cultures throughout the period of observation of the tooth. Reiman and Havens (2) also mentioned this phenomenon. We think that Buchbinder and Wald (3, 4) would hold to this same idea. Buchbinder and Wald of course are actively doing something about it in anaerobic studies.

In a paper presented by us (5) last year we got the following results using our routine aerobic method of cultivation: In a group of 59 cases, all designated as showing a circumscribed radiolucent reaction by original roentgenograph, 31 (53 per cent) showed no organisms at any time, while 15 (25 per cent) showed single organism infection, and we got mixed infection in 13 (22 per cent). In the large group of cases, (all followed for from 1 to 7 years), which gave a diffuse radiolucent reaction, we found that we had a total of 170 followed-up cases and of these 33 (19 per cent) gave no organisms at any time; 24 (15 per cent) gave single organism infection; and 113, (66 per cent) showed a mixed infection. We do not wish at this time to draw any comparisons between the circumscribed and the diffuse group, although it seems to us that there are some distinct differences other than roentgenographic variation.

In a group, spoken of by us as normal or showing no roentgenographic change, 10 of 28 cases (36 per cent) showed no organism; and 4 (14 per cent) showed single organism infection, and 14 (50 per cent) mixed infection.

These were the results, in a total of 257 cases, followed-up for a very considerable time following the placing of the root canal filling. Only 7 per cent of cases failed to show improvement as far as evidence by radiograph could determine this state. Our point is that we found no organisms in a great many cases.

From previous experience using anaerobic cultures we have felt that possibly anaerobes which failed to grow in our ordinary media were the cause for this discrepancy in theoretical and practical results. In the past we have tried occa-

[1] Presented at the 19th General Meeting of the International Association for Dental Research, St. Louis, March 15–16, 1941. (*J. D. Res.* **20**: 249, 1941.) Received for publication August 5, 1941.

sionally to use anaerobic methods, but in a routine procedure which concerns several individuals and at times a change in individuals because different men have to carry on the root canal clinic and different assistants have to carry on the cultivation, we had found that ordinary anaerobic procedures using extensive apparatus of jars and either chemicals or vegetables or other methods for exhausting the oxygen did not give us consistent results.

Recently, however, Brewer (6) described a method of anaerobic cultivation using sodium thioglycollate as an oxidation reduction agent in the media using a slight amount of agar to prolong the anaerobic effect. In this media it was possible to use a 1 to 500,000 dilution of methylene blue as an indicator of the anaerobic state. The methylene blue was non-toxic in this dilution and gave no bacteriostatic effect. This media also tended to inactivate mercurials which might be used as preservatives in testing of catgut and other substances which may carry anaerobes of danger in surgical procedures. This type of media, according to Brewer, will remain anaerobic for at least a month, treated in the way one would ordinarily treat aerobic types of media. This can be obtained in dehydrated form.

McClung (7, 8) tested Brewer's medium using decimal dilutions of cultures of clostridia and found that with the dehydrated thioglycollate media, or with a liver infusion broth with thioglycollate, he got as good or better results than with any other anaerobic method used as a control.

We adopted some of these media to see whether they would work satisfactorily in root canal procedure; and in addition to our standard media (a 1 per cent dextrose pH 7.6 broth which we have used for years in our root canal studies), as a control, we added dehydrated thioglycollate media, a 1 per cent dextrose infusion thioglycollate medium, a 0.5 per cent dextrose infusion thioglycollate medium, and dehydrated brain and heart infusion without thioglycollate. The procedure has been to have the root canal operator inoculate with sterile paper points the test media and the control media as in our usual practice (9).

These cultures are incubated for the first 24 hours in the usual fashion. After that, they are incubated in an anaerobic jar with chromium powder plus 50 per cent sulphuric acid as the means to establish a state of anaerobiosis, using methylene blue tubes to check on the effectiveness of the reactions. The jar anaerobic cultivation is continued for 2 or 3 more days, awaiting growth in the tubes if any will develop in that time.

Positive tubes are planted on streak plates of blood agar and these are grown both aerobically and anaerobically in duplicate. The results are shown in Table I. Anaerobic cultures were made on 4 different media. The control cultures were made in duplicate, one being taken from the old dressing, and the other by means of a fresh sterile paper point. A difficulty arises when attempting to take several samples from a root-canal in that the material in the canal may be exhausted by continued culturing with paper points. In order to test the 4 media mentioned and the control media, 6 samples were taken from each root canal.

The total number of anaerobic cultures taken was 153, of which 43 were posi-

tive and 110 negative. There was very little difference between the types of anaerobic media used, as shown by the number of positive cultures obtained. The third type, a 0.5 per cent dextrose infusion broth containing 0.1 per cent sodium thioglycollate gave 13 positive cultures as compared with 10 positive cultures each for the other 3 types of anaerobic media. In making the comparison on the 44 teeth examined we have grouped all the anaerobic cultures together, and the aerobic controls together. If one culture was positive, the root canal was considered to show a positive culture in that class of media. The anaerobic cultures showed 16 positive cases and 28 negative in a series of 44 teeth investigated. On the other hand the aerobic cultures showed 22 positive and 22 negative cases, or the aerobic cultures gave more positive cases. On further comparison we found that we had positive anaerobic and positive aerobic (control) cultures in 14 cases, and negative anaerobic with negative aerobic (control) cultures in 20 cases. The remaining 10 cases gave results which showed a disagreement between the anaerobic and aerobic cultures. Negative

TABLE I

DUPLICATE CULTURES OF CASES	RESULTS	
	Pos.	Neg.
Anaerobic	16	28
Aerobic (controls)	22	22
Anaerobes + Controls +	14	
Anaerobes − Controls −	20	
Anaerobes − Controls +	7	
Anaerobes + Controls −	3	

Total Anaerobic Cultures 153—Pos. 43—Neg. 110

anaerobic cultures with positive aerobic cultures appeared in 7 cases, while, positive anaerobic cultures with negative aerobic control cultures occurred in only 3 cases.

In considering the types of organisms which might appear on cultivation in these different media, we found that 8 of the positive cases gave the alpha or viridans type of streptococcus, while the gamma type of streptococcus appeared in 3 cases. Aciduric bacilli appeared in 4 and staphylococcus albus in two. Mixed cultures occurred 3 times, once with aciduric bacilli plus *Staphylococcus albus*, and twice with *Streptococcus viridans* and *Staphylococcus albus*.

CONCLUSIONS

Sodium thioglycollate media as a method for the anaerobic development of root-canal cultures does not give us an increase in the number of positive cultures obtained from a given number of cases. The negative culture so often obtained on the first opening of a dental root canal probably may be expected, and is not due entirely to inadequate bacteriological methods.

SUMMARY

Anaerobic methods utilizing media containing sodium thioglycollate were applied to bacteriologic cultures of dental root canals. Fewer positive cultures were obtained than by means of our customary aerobic medium. The best anaerobic results were obtained with a medium containing 0.5 per cent dextrose in an infusion broth with 0.1 per cent sodium thioglycollate.

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POST-MORTEM BACTERIOLOGIC STUDIES OF DIFFERENT AREAS OF HUMAN TEETH AND THEIR SUPPORTING STRUCTURES

AN ATTEMPT TO TRACE THE POSSIBLE PATH BY WHICH BACTERIA REACH THE PERIAPICAL REGION OF INTACT TEETH[1]

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Bacteria have been demonstrated previously about the apexes of 30 per cent of all teeth without periapical radiolucent areas (1). This paper deals with the bacteriologic findings obtained from the blood stream, the gingival crevice, periapex, root apex, coronal pulp and dental periosteum midway between the apex and marginal gingiva of 54 human teeth obtained at 13 different necropsies. The possibility of organisms gaining access to the periapical region by way of the blood stream, dental periosteum and the dentin and dental pulp in the case of carious teeth was suggested by a study made previously on post-mortem material.

As early as 1878, Rogers (2) suggested the possibility of the hematogenous route in periapical lesions. Later Rickert (3) discussed the possible significance of passive bacteremias to periapical lesions. Henrici and Hartzell (4) suggested the vascular channels as an avenue of infection in explaining the pulpal and periapical abscesses in teeth with sound enamel. More recently Itakura (5) has demonstrated the leprosy bacillus in the pulpal tissue of supposedly intact teeth.

The study of Lazarus-Barlow (6) offers suggestive evidence as to how organisms may reach the periapical region. He obtained post-mortem cultures from 516 human teeth during a bacteriologic study of "pyorrhea." Material was removed at 3 different levels; 3 mm. below the root apex, at the root apex, and 3 mm. below the gingival margin, by drilling through the buccal cortical bone with aseptic precautions. Positive cultures were obtained in 20 per cent of the teeth examined. The majority of the positive cultures was secured from material obtained 3 mm. below the gingival margin, 6 times from the periapical region and 3 times from the bone beyond the apex. *Streptococcus viridans* (alpha streptococcus) was the organism most frequently isolated.

While Lazarus-Barlow's results might be interpreted as emphasizing the importance of the dental periosteum as an avenue by which bacteria reach the tooth apex, several points must be borne in mind before any deductions are

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made from these findings. Gross or roentgenologic examinations were not made of the teeth studied, and since the exact site of the culture was not visualized there is no assurance that the material for bacteriologic study was obtained from the root apex or 3 mm. below the gingival margin. Furthermore the entire head of the dental bur, which contained material collected while passing through the submucosal tissues and alveolar bone, was incubated.

Rickert (7) found that the bacteriologic flora of the periapical region differed from that of the apical portion of the pulp. Furthermore he stated that in contrast to the rather common occurrence of bacteria about the apexes of teeth their presence in the substance of the alveolar bone was a rarity.

The importance of the blood stream as an avenue by which bacteria may reach the periapical region has not been sufficiently appreciated. Recent studies have disproved the belief that transient bacteremias are infrequent and evidence has accumulated from a wide variety of studies pointing to their rather common occurrence (8, 9). These studies have varied from bacteremias demonstrated after tonsillectomies (10) or submucous resections to those existing in menstruating women (11). Bacteremias have been demonstrated in individuals with chronic arthritis (12, 13) and after a change in the diet (14, 15). Transient bacteremias have also been demonstrated following the extraction of teeth under inhalation (16) or local (17) anesthesia. Irritation of a foci of infection is also known to produce a transient bacteremia (18, 19). Experimental bacteremias have been produced in dogs by the removal of infected teeth (20).

It has been shown experimentally by Bereta (21) and Hill (22) that bacteria in the blood stream localize frequently about the apices of teeth. They have been observed to persist in this region when they were no longer demonstrable in the blood stream or other viscera. This has also been shown to be true in the case of man (23). The more recent work of Czernyei (24) and Robinson and Boling (25) on the anachoretic effect emphasizes further the importance of the hematogenous route. In view of this knowledge and the evidence pointing to the common occurrence of bacteremias, it is possible that the blood stream may play a significant role in explaining the presence of bacteria about the apices of clinically and roentgenologically negative teeth.

MATERIAL AND METHODS

Post-mortem material was used for the present study. Since the culturing technic was limited in its application to single rooted teeth, only specimens of the anterior maxilla were removed. The general methods of obtaining the specimens, the gross and roentgenologic examinations were similar to those previously employed (26). The culturing technic and the types of culture media used differed. Burn (27) has described the methods used in securing the post-mortem blood stream and visceral cultures.

In the bacteriologic laboratory the specimen was mounted on a small wooden block by means of dental impression compound. The block was clamped in a bench vise. Cultures of the gingival crevice were first obtained with a steril No. 18 excavator. Concentrated iodine solution in 70 per cent alcohol was then applied several times to the gingival crevice and buccal mucosa after which these tissues were removed exposing the cortical bone, except for a narrow band of soft tissues immediately surrounding the gingival margin of the tooth. The same sterilizing agent was then painted on the cortical bone and cultures were taken to check on the sterility of this surface.

A small window was then cut in the cortical bone in the apical region, with a steril No. 700 cross-cut fissure bur. After the dense bony plate was pried out by means of a steril dental excavator, the surface of the exposed root was scraped vigorously to obtain material for culture. The apical tip of the root was then cut off with another steril bur, removed from the specimen, and dropped into the culture medium by means of college pliers. Another small window was then cut in the cortical bone midway between the apex and the marginal gingiva, exposing the root surface in this region. A small "V" shaped piece of root substance and dental periosteum was obtained and dropped into a tube of culture medium. Pulpal cultures were secured by physically cleansing the crown of the tooth and then painting it several times with iodine solution after which a steril carborundum wheel was used to penetrate the enamel in a portion of the crown that was free from caries and distant from the gingival tissues. A steril No. 1 round bur was used to enter the pulp chamber, allowed to lacerate a portion of this structure while it was revolving slowly, then removed and the head of the bur broken off with steril pliers and dropped in the culture medium.

Holman's cooked ground meat medium was used for all the innoculations. All cultures were examined at the end of 24 and 48 hours. Negative cultures were incubated for 7 days. All tubes were plated out on tomato-agar (pH 4.5) and surface agar plates to which 5 per cent defibrinated rabbit's blood had been added. Smears, stained with Gram's stain and gentian violet were made from the cultures and examined.

The above outlined procedures then included cultures taken from:

1. The gingival crevice prior to study
2. The buccal cortical bone after sterilization
3. The periapex of the root.
4. The apical tip of the root with the contained pulp tissue.
5. The small triangular piece of root and dental periosteum obtained approximately half way between the gingival margin and the apex.
6. The pulp tissue and
7. The blood stream and viscera.

In addition to the technic outlined above, additional experimental procedures and cultures were made in 7 of the cases studied. The use of *Serratia marcesens*, as a bacteriologic control of the technical methods was suggested by a clinical study concurrently in progress (17). A heavy suspension of *S. marcesens* was painted about the gingival margin prior to the removal of the specimen from the body, or at varying intervals of time before culturing was begun.

DATA

Fifty-four teeth and 8 areas in the alveolar bone, where teeth had been previously extracted, were examined. Two teeth with exposed pulps had definite periapical radiolucent areas but roentgenologic changes were not noted about the apices of the remaining teeth. These teeth, with two exceptions, were grossly free from carious or presented minimal carious lesions. Varying degrees of alveolar resorption were present. None of the teeth were frankly loose.

The location and sequence of the areas cultured are shown in *fig. 1*. The frequency of the positive cultural results are summarized in Table I. The usual mouth flora organisms were obtained in every instance from the gingival crevice cultures before the use of iodine. Twelve periapical areas (22 per cent) and the root apices of 25 teeth (46 per cent) also yielded bacteria. Bacteria (alpha streptococci) were recovered once from the root fragments taken approximately half way between the apex and marginal gingiva. Three positive pulpal cultures were recorded. Two of these were from teeth with exposed pulps—beneath fillings. A diphtheroid was isolated from the other pulp. With one exception all the cortical bone cultures were negative.

The periapical cultures obtained by scraping the dental periosteum of the root surface, and the apex cultures which included the apical tip of the root and the contained pulp tissue were studied in detail. Although the number of positive growths is small, the relative frequency of the organisms recovered from these two regions has been compared in Table II. A comparable bacterial flora was observed in this small group for the root apex with the contained pulp

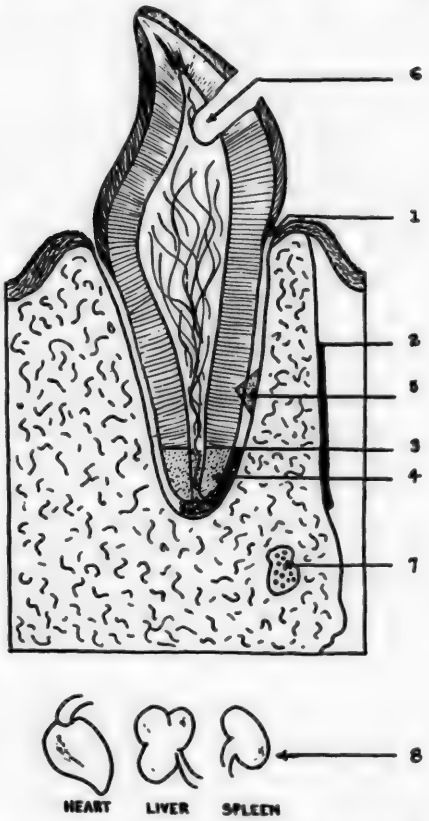

FIG. 1. Location and sequence of areas cultured. 1—gingival crevice, 2—cortical bone (control), 3—periapex, 4—apical tip with contained pulp, 5—triangular root piece including dental periosteum, 6—coronal pulp tissue, 7—marrow substance at site of previous extractions, 8—heart's blood and visceral cultures.

tissue, and for the periapical cultures. Pure growths were obtained in 64 per cent of the periapical and 33 per cent of the apex examinations. Only one periapical culture yielded growth without a similar result in the root apex examination; however, 13 apex cultures were positive without a corresponding periapical growth. Similar types of organisms were observed in both the periapical and apical cultures of the remaining teeth. The similarity of the organisms was judged by their morphologic characteristics and their hemolytic activity on

surface agar plates and not by serologic or biochemic studies. All 8 cultures of the alveolar marrow substance at the site of previous dental extractions were steril.

The post-mortem blood and visceral findings were studied in relation to the dental cultures. Organisms were grown from the blood stream, liver or spleen cultures in 4 necropsies. From a case whose clinical course suggested an acute septicemia, at necropsy a *Staphylococcus aureus* with marked hemolytic activity was cultured from the spleen and 2 periapical regions although the clinical and

TABLE I

Frequency of positive cultures obtained from the different areas of the teeth and their supporting structures

	AREA	NO. CULTURES	NO. POSITIVE CULTURES INCLUDING *S. marcesens*	PER CENT POSITIVE
1	Gingival crevice	54	54	100
2	Cortical bone	54	1*	2
3	Periapex	54	12	22
4	Apex with apical pulp	54	25	46
5	Triangular root piece with dental periosteum	54	2	4
6	Coronal pulp	54	3**	6
7	Marrow substance	8	0	0

* Diphtheroid organism similar to organism recovered from blood and viscera.

** One positive culture for diphtheroid. Diphtheroids were also cultured from 4 periapexes and 2 apex cultures in this same case.

TABLE II

Frequency in per cent of the organisms recovered from the periapical and apex cultures

ORGANISM	PERIAPEX	APEX WITH CONTAINED PULP
Streptococcus viridans (alpha streptococcus)	66	64
Streptococcus non-hemolyticus	25	20
Streptococcus hemolyticus	0	8
Staphylococcus aureus	58	44
Diphtheroid	8*	4*
Gram negative coccus	8	8

* A similar organism was recovered from the heart's blood, viscera, cortical bone and coronal dental pulp.

post-mortem blood cultures and 3 other periapical cultures showed no growth. The roentgenograms revealed no pathologic changes about the apices of the 2 teeth yielding growth that would distinguish them from the 3 yielding no growth.

An anerobic Gram-negative rod was isolated from the liver and a *Staphylococcus aureus* was obtained from the spleen of another case. Although a staphylococcus was obtained from the apical region of several of the teeth their cultural characteristics were not similar enough to permit any association of the organisms found in the teeth and spleen.

In a case of subacute bacterial endocarditis in which the symptoms of onset were definitely related to the removal of an abscessed tooth, the blood stream during the clinical course of the disease and at necropsy was positive for *Streptococcus viridans* (alpha streptococcus). This organism was isolated from the periapex, the apical portion of the root and the dental pulp. Differences were observed in the serologic findings of the organisms recovered from the blood stream and the dental pulp.

S. marcesens was used as a bacteriologic control for the technical culturing procedures in 7 of the 12 cases. Thirty teeth were examined from this group. Growths containing *S. marcesens* were obtained from 6 teeth in 2 cases. Five of these were obtained from a single case in which death had occurred more than 24 hours before the periapical bacteriologic studies were begun. Post-mortem changes in the tissues, especially the dental periosteum, may have been a factor in addition to the marked degree of alveolar atrophy exhibited by this case. On gross examination the second case showed a marked attrition and a moderate degree of mobility of the teeth. When the gingival tissues were dissected free it was noted that the alveolar bone surrounded only the apical third of the roots. *S. marcesens* was obtained from one of the cultures made approximately midway between the apex and the marginal gingiva—actually 2 mm. from the alveolar crest.

COMMENT

In this study the periapical cultures consisted of scrapings from the apical portion of the dental periosteum while those from the apex included the terminal 2–3 mm. of the root with its periosteal covering and also the contained pulp tissue. The similar bacteriologic findings in the root tip and periosteal cultures established the adequacy of the technic used in previous studies for bacteriologic examination of the periapical region (26).

The low incidence of positive pulpal cultures is of significance when compared with the relatively high incidence of growths obtained by others from the pulpal tissue of extracted teeth (28, 29, 30, 31, 32, 33, 34, 35). Since the pulp cultures were taken with the teeth in situ it obviated the possibility of organisms being mechanically forced into the pulp during extraction. As a result, the findings probably more nearly represent the actual occurrence of bacteria in the pulp of non-carious teeth and those with minimal carious lesions. They are in agreement with the bacteriologic findings made on living pulps of teeth in situ prior to extraction (36). Although media were used which permitted the growth of aciduric bacteria they were not demonstrated in the pulp or other dental tissues.

S. marcesens was used to determine whether the trauma incident to the removal of the specimen from the body forced organisms into the periapical tissues from the gingiva crevice. A suspension of this organism was painted about the gingiva prior to the removal of the tissue. The control organism was only recovered from the gingival crevice, except in the tissue examined more than 24 hours after death. Here the *S. marcesens* organisms were also isolated from the periapical and root apex cultures but not the coronal pulp. This suggested

the possibility that invasion of a post-mortem nature had occurred through the dental periosteum. This may have been due to some sort of post-mortem change, the motility of the organism or the moderate degree of alveolar resorption present in this case. The fact that 78 cultures made from different regions of the dental structures were negative for *S. marcesens* minimizes the possibility of the dental bacteriologic findings being affected by the source of material employed or by the technical procedures used in removing or culturing the tissues. Cultures were usually made within the first few hours after death.

The dental periosteum played an unimportant role in the periapical bacteriologic findings, as judged by the cultural results. The apparently steril condition of this tissue may be due to errors in sampling although comparable amounts of dental periosteum were cultured from the periapical region and the root surface midway between the apex and marginal gingiva. Again the inability to demonstrate organisms in the dental periosteum does not negate its importance as an avenue by which bacteria may reach the periapical region during life.

The anatomical difference between the marginal gingiva and the margin of the alveolar bone was striking. This explains why some cultures were actually taken within 2 to 3 mm. of the alveolar crest when they were made midway between the root apex and the marginal gingiva. The positive growths of *S. marcesens* and *Streptococcus viridans* were obtained from the dentin and dental periosteum removed 1.5 mm. and 2 mm. respectively from the alveolar crest. The cultures were usually made 2.5 mm. to 3 mm. from the alveolar crest. These all failed to yield growth. These tissues were not observed to have been discolored by the sterilizing solutions used to prepare the cortical bone before culturing.

The significance of the blood stream in explaining the presence of bacteria in the periapical region can only be partly evaluated by post-mortem studies. In two cases of this small series it seemed likely that organisms recovered from the apical region could be explained by a bacteremia which was previously demonstrated. It is known that clinical bacteremias may be responsible for post-mortem periapical findings although the particular bacterium can not be demonstrated in the blood stream or viscera at necropsy (23).

A realization of the common occurrence of transient bacteremias following a wide variety of conditions, places increased emphasis on the vascular avenue of periapical invasion, especially when it is known that for some anatomic or physiologic reason, organisms or even perhaps particulate material have a tendency to localize about tooth apices. The anachoric effect in the periapical region may also play a part.

SUMMARY

Post-mortem cultures taken from different areas of 54 human teeth in situ were compared with the bacteriologic studies of the blood stream and viscera. A similarity in respect to the frequency of positive growths and the types of organisms recovered was observed in the cultures from the periapex and the root apex with its contained pulp. Examinations of the coronal pulp and dental

periosteum obtained midway between the apex and marginal gingiva rarely yielded bacteria.

While the results of these few observations permit no definite conclusions as to the avenue by which bacteria may gain access to the periapical region of teeth without marked caries or periodontoclasia, suggestive evidence points to the blood stream as being a factor of significance.

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BLOOD FINDINGS ON TWENTY PATIENTS BEFORE AND AFTER EXTRACTION OF TEETH[1]

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Pain, discomfort, and complications that patients frequently experience following the removal of teeth, have been distressing to the dentist over a long period of time. Even though much progress has been made in a clinical manner for the control of pain in these cases, dry sockets and bone infections which endanger the health, and sometimes the lives of patients, still persist. It would be of benefit to both patient and dentist, if a method could be devised to avoid these unfortunate occurrences.

Systemic effects due to periodontal infection have been observed for some time, by Hunter (1900), Goadby (1911), Billings, Davis, Nicol, Rosenow, and Liebman (1912) and Hartzell (1914). Logan (1915) was perhaps one of the first to observe leucopenia and leucocytosis associated with varying degrees of periodontal and periapical pathosis (3).

Transient bacteremia following the extraction of teeth was first reported by Okell and Elliott in 1935 (1). Fish and MacLean in 1936 reported similar observations which were considered the result of the pumping action created during the extraction of a tooth. They also mention Round, Kilpatrick, and Hails reporting transient bacteremia as the result of the chewing of hard candy in patients who had oral sepsis (2).

Andey and Ferguson reported a leucopenia and a leucocytosis noted in blood findings from albino rats and rabbits in whom teeth had been extracted. They further stated that such a condition exists in the form of a diphasic curve; that is, first a transient leucopenia and then a leucocytosis, which was peculiar in animal experiments following the extraction of teeth (4).

It is an accepted fact that leucocytosis may be produced by merely pinching, cutting, or otherwise traumatizing the tissues of the body. In the author's investigations this transient leucopenia, followed by a leucocytosis, has been observed following the extraction of teeth in man.

The following report represents blood findings on 20 patients before and after extraction of teeth.

OBSERVATIONS

I.[2] Nine cases in which blood examinations were made after extractions, at hourly intervals for 6 consecutive hours, gave the following result:

A. A leucopenia at the end of 1 hour in 4 out of 9 cases (Cases 4, 6, 8, and 9).

[1] Received for publication August 24, 1941.

[2] The use of an electric cautery loop around the gingival attachment of the tooth prior to extraction as suggested by Fish was not used in the first 9 cases.

B. A leucocytosis at the end of 1 hour in 5 out of 9 cases (Cases 1, 2, 3, 5, and 7).

C. Blood cultures before the extractions were all found to be negative.

D. Blood cultures after the extractions were found to be negative in 8 of the 9 cases after 2 hours. 1. Cultures negative after 6 hours in all cases. 2. Culture positive in 1 case (Case 8), 2 hours after extraction, then negative at the end of 6 hours.

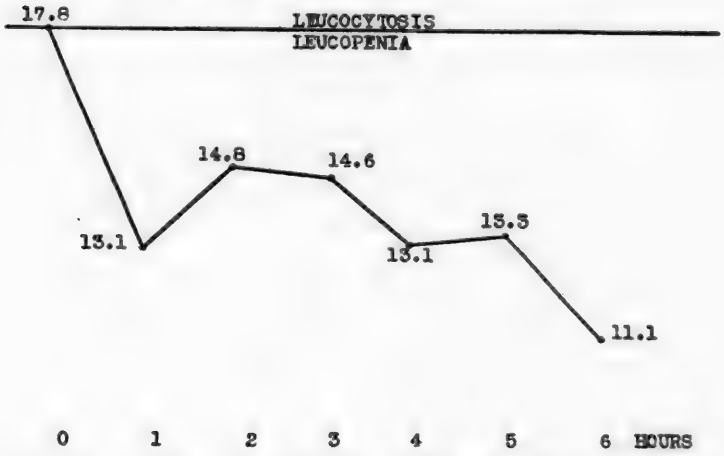

FIG. 1. Case #4. White blood cell count in thousands per c.mm.

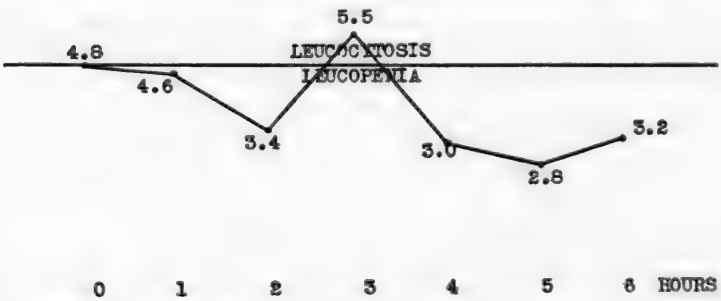

FIG. 2. Case #6. White blood cell count in thousands per c.mm.

E. Bacteriological findings of all root apices cultured, revealed predominating *Streptococcus viridans*.

F. Sedimentation tests revealed nothing of importance.

II. A study of the production of leucopenia, including white blood cell count, differential count, and polymorphonuclear percentages, with a report of the patient's health history revealed the following:

A. Case #4: Patient has previous history of tonsilitis and duodenal ulcer. 1. Findings before extraction: a. White blood cells: 17,800 per c.mm. (*fig. 1*); b. Differential count: Lymphocytes—40 per cent; Polymorph. leukocytes—Segs. 60 per cent. 2. Findings after

extraction: Polymorphonuclear leukocyte percentages in 6 consecutive examinations: 78, 70, 94, 84, 80, and 74 per cent. Average for 6 hours, 80 per cent.

B. Case #6: Patient is a chronic alcoholic. 1. Findings before extraction: a. White blood cells: 4,800 per c.mm. (*fig. 2*); b. Differential count: Lymphocytes—79 per cent; Polymorph. leukocytes—Segs. 21 per cent. 2. Findings after extraction: a. Polymorphonuclear leukocyte percentages in 6 consecutive examinations: 23, 33, 22, 24, 30, and 40 per cent. Average for 6 hours, 29 per cent.

C. Case #8: Patient has diabetes mellitus. 1. Findings before extraction: a. White blood count: 8,200 per c.mm. (*fig. 3*); b. Differential count: Lymphocytes—50 per cent; Monocytes—2 per cent; Eosinophiles—1 per cent; Polymorph leukocytes—Segs. 47 per cent. 2.

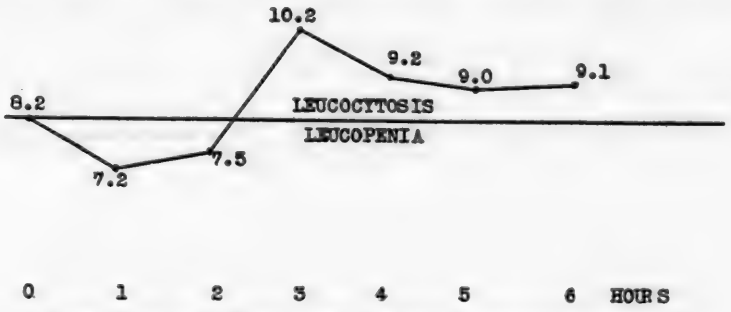

FIG. 3. Case #8. White blood cell count in thousands per c.mm.

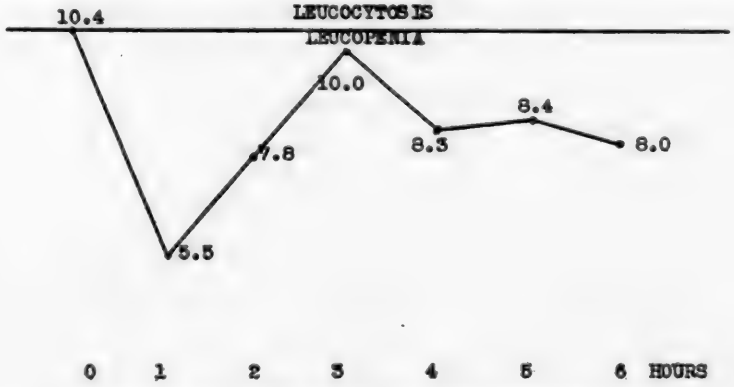

FIG. 4. Case #9. White blood cell count in thousands per c.mm.

Findings after extraction: a. Polymorphonuclear percentages in 6 consecutive examinations: 52, 65, 56, 74, 64, and 50 per cent. Average for 6 hours, 60 per cent.

D. Case #9: Patient has diabetes mellitus, and has had a general arteriosclerosis, with hypertension and myocardial insufficiency, and a low grade inflammation of the left foot. 1. Findings before extraction: a. White blood cells: 10,400 per c.mm. (*fig. 4*); b. Differential count: Lymphocytes—30 per cent; Monocytes—2 per cent; Eosinophiles—3 per cent; Polymorph. leukocytes—Segs. 65 per cent. 2. Findings after extraction: a. Polymorphonuclear percentages in 6 consecutive examinations: 56, 60, 51, 64, 74, and 62 per cent. Average for 6 hours, 61 per cent.

E. Average total increase of polymorphonuclear leukocytes in 4 cases—10 per cent.

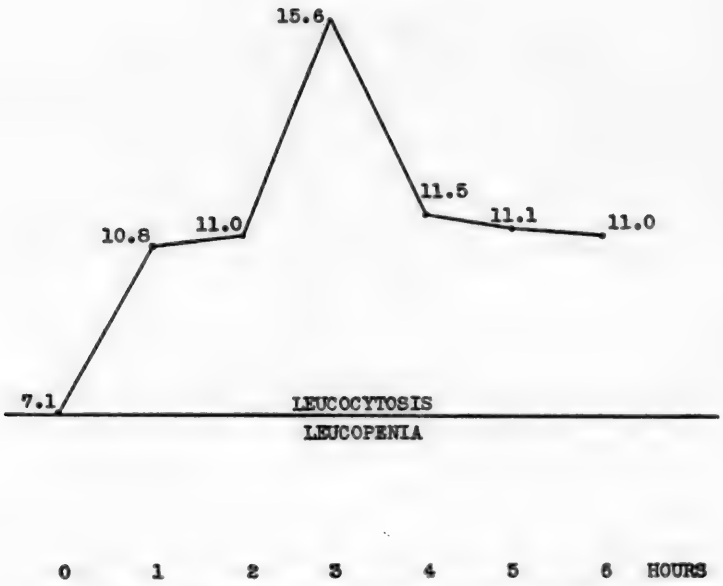

FIG. 5. Case #1. White blood cell count in thousands per c.mm.

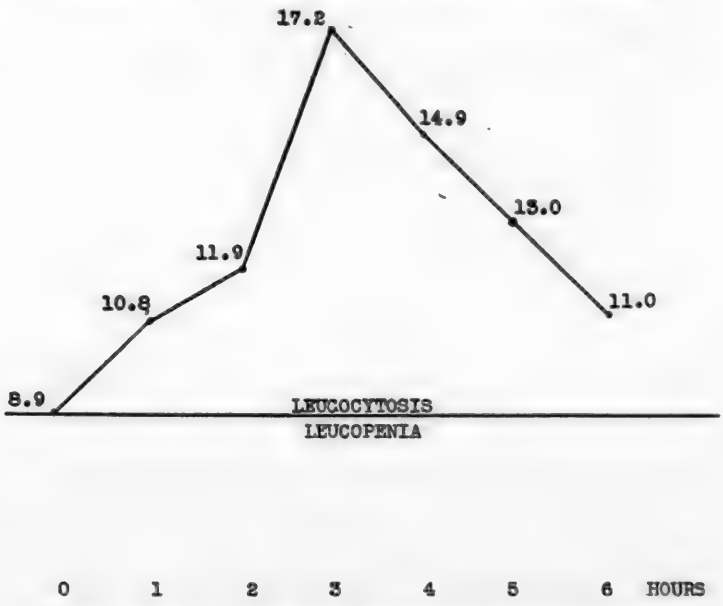

FIG. 6. Case #2. White blood cell count in thousands per c.mm.

III. A study of the production of leucocytosis, included white blood cell count, differential count, and polymorphonuclear leukocyte percentages, with a report of the patient's health history.

A. Case #1: Patient has severe sciatica, with subacute arthritis in the lumbar spine. 1. Findings before extraction: a. White blood cells: 7,100 per c.mm. (*fig. 5*); b. Differential count: Lymphocytes—34 per cent; Monocytes—2 per cent; Eosinophiles—1 per cent; Polymorph. leukocytes—Segs. 60 per cent and Stabs. 3 per cent. 2. Findings after extraction: Polymorphonuclear percentages in 6 consecutive examinations: 54, 62, 81, 70, 65, and 61 per cent. Average for 6 hours, 66 per cent.

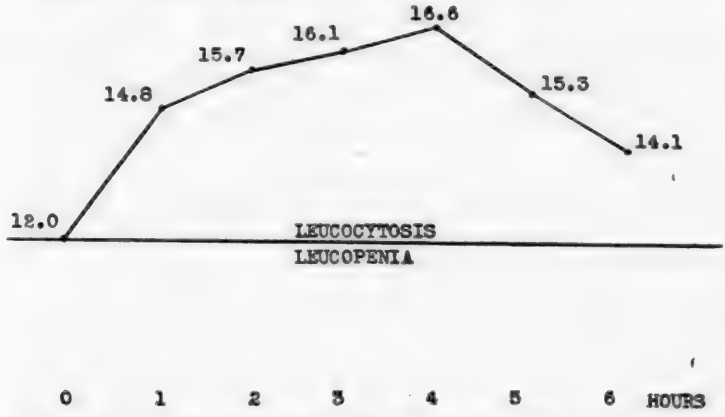

FIG. 7. Case #3. White blood cell count in thousands per c.mm.

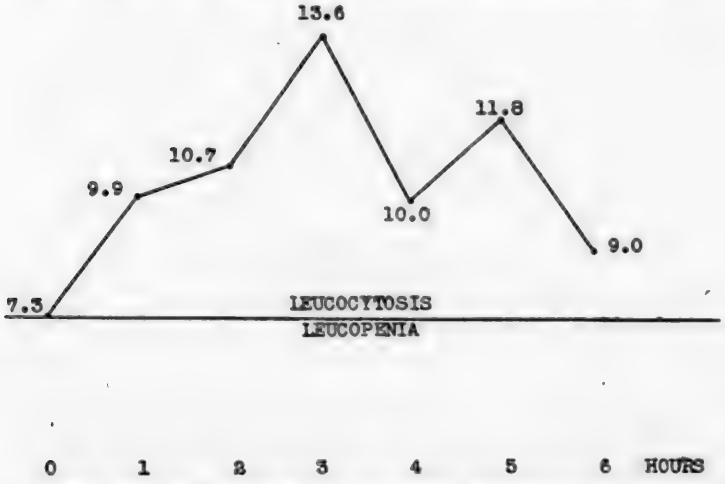

FIG. 8. Case #5. White blood cell count in thousands per c.mm.

B. Case #2: Patient has ulcerative colitis. 1. Findings before extraction: a. White blood cells: 8,900 per c.mm. (*fig. 6*); b. Differential count: Lymphocytes—42 per cent; Polymorph. leukocytes—Segs. 58 per cent. 2. Findings after extraction: a. Polymorphonuclear percentages in 6 consecutive examinations: 79, 81, 78, 73, 80, and 78 per cent. Average for 6 hours, 78 per cent.

C. Case #3: Patient was suffering from peptic ulcer at the time of the investigation. 1. Findings before extraction: a. White blood cells: 12,000 per c.mm. (*fig. 7*); b. Differential count: Lymphocytes 40 per cent; Polymorph. leukocytes—Segs. 60 per cent. 2. Findings

after extraction: Polymorphonuclear percentages in 6 consecutive examinations: 72, 76, 79, 78, 74, and 77 per cent. Average for 6 hours, 76 per cent.

D. Case #5: Patient was suffering from duodenal ulcer and thyroid adenoma at the time of the investigation. 1. Findings before extraction: a. White blood cells: 10,400 per c.mm. (*fig. 8*); b. Differential count: Lymphocytes—20 per cent; Monocytes—4 per cent; Polymorph. leukocytes—Segs. 76 per cent. 2. Findings after extraction: Polymorphonu-

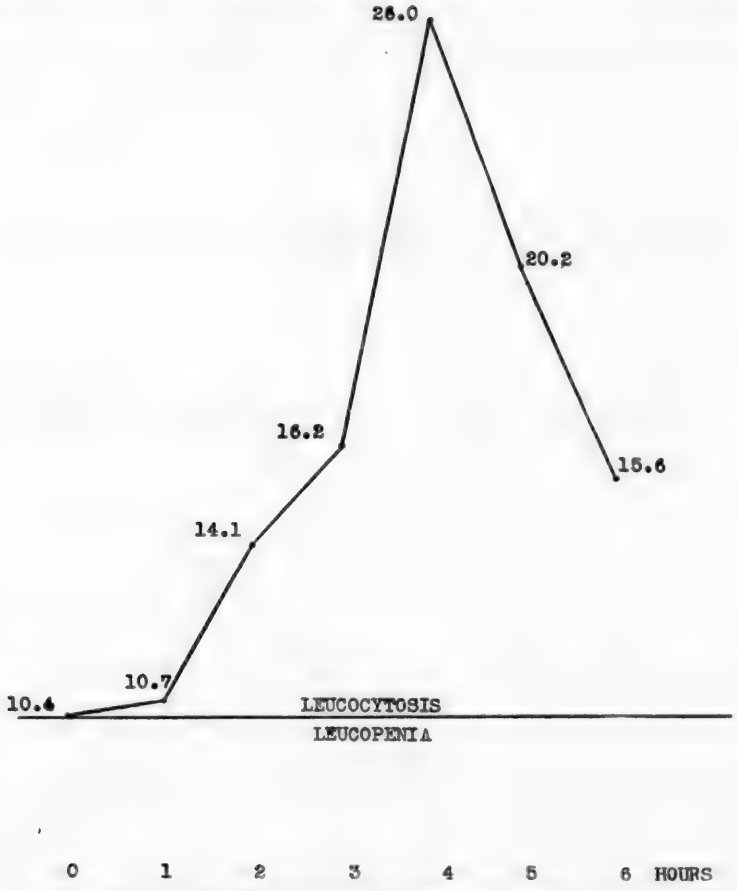

FIG. 9. Case #7. White blood cell count in thousands per c.mm.

clear percentages in 6 consecutive examinations: 78, 90, 87, 82, 86, and 92 per cent. Average for 6 hours, 86 per cent.

E. Case #7: Patient was suffering from chronic fibrous pleurisy at the time of the investigation. 1. Findings before extraction: a. White blood cells: 7,300 per c.mm. (*fig. 9*); b. Differential count: Lymphocytes—52 per cent; Polymorph. leukocytes—Segs. 48 per cent. 2. Findings after extraction: Polymorphonuclear percentages in 6 consecutive examinations: 46, 64, 55, 73, 76, and 42 per cent. Average for 6 hours, 58 per cent.

F. Average total increase in 5 cases in polymorphonuclears, 13 per cent.

IV. Eleven cases in which blood was examined immediately after extractions and for 6 consecutive hourly periods gave the following:

A. A leucopenia was noted immediately after extraction in each of 10 cases out of 11 (Cases 10, 11, 12, 13, 15, 16, 17, 18, 19, and 20).

B. A leucocytosis was noted immediately after extraction in 1 case (Case 14).

C. A leucopenia of 1 hour duration was observed in each of 10 cases.

D. A leucopenia lasting for 6 hours was present in 1 case (Case 16).

E. A leucocytosis preceded by leucopenia was found in each of 10 cases.

F. Blood cultures were found to be negative in all cases at the beginning of the investigations.

G. Blood cultures were found to be positive immediately after extraction in 2 cases (Cases 10 and 12); 1 hour after extraction in 4 cases (Cases 15, 17, 19, and 20); 2 hours after extraction in 3 cases (Cases 10, 13, and 19); 3 hours after extraction in 1 case (Case 18) and 6 hours after extraction in 1 case (Case 18, slow-growing organism).

H.[3] Cultured root apices gave the following bacteriological results: 1. Positive cultures in 4 cases out of 11 (these growths were scanty, might have been due to contamination).

V. A study of the production of leucopenia in 11 cases included white blood cell count, differential count, and polymorphonuclear leukocyte percentages, with a report of the patient's health history.

A. Ten cases responded in the same manner as the preceding 9 cases; that is, rises in polymorphonuclear leukocyte percentages were observed durthe investigation.

B. The unusual and outstanding case (Case 14), gave only a condition of leucocytosis following extraction, resulting in a monophasic curve instead of the usual and expected diphasic curve. This patient has a carcinoma of the lip.

CONCLUSIONS

1. From the findings it is concluded that the extraction of teeth is followed by immediate leucopenia, which is subsequently followed by a leucocytosis.

2. Extraction of teeth is associated with a general disturbance of the blood as proven by the blood findings.

3. In 40 per cent of the cases studied, bacteremias were observed. This fact brings out the importance of proper evaluation of physical conditions and preparation of the patient who is subjected to the extraction of teeth.

4. The use of the electric cautery about the gingival soft tissues of extracted teeth was found to be of benefit. This was established by the fact that in most instances, root apices of such extracted teeth were found to be negative upon culture for bacteria.

[3] All of the root apices cultured represented teeth in which the gingival attachment had been cauterized with an electric cautery loop.

The author wishes to acknowledge the cooperation of the United States Veterans Administration and the following men of the Veterans Administration who participated in these investigations: Dr. Lewis Nolan, Pathologist; Dr. Clarence S. Lister, Chief Dental Officer; Dr. C. Logsdon, Oral Surgeon; and Mr. Q. Polanco, Clinical Expert Laboratorian.

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THE HISTOPATHOLOGY OF THE INCISOR TEETH OF MICE WITH EXPERIMENTALLY PRODUCED TUBERCULOSIS[1]

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References to experimental studies dealing with the relationship of tuberculosis to tooth development have not been found in the literature, but that this disease may be an etiologic factor in enamel hypoplasia has been suggested. Zsigmondy (1) mentioned "scrofulosis" as an alleged cause. Neumann (2) also described "circular caries" of the deciduous teeth which he believed was most frequently associated with scrofula and tuberculosis.

In the present study, the incisor teeth of mice with experimentally produced tuberculosis were examined and definite changes observed in the ameloblasts and subjacent surface of organic enamel matrix. These lesions closely resemble those described by the author (3) in human material as representing the earlier stages in the pathogenesis of enamel hypoplasia.

Similar abnormalities of tooth development, attributed to a wide variety of causes, have been reported. Erdheim (4) and Toyofuku (5) produced the lesions in rats by parathyroidectomy. Ameloblastic injury and abnormality of the enamel matrix were also noted by Mellanby (6) in puppies fed a rachitogenic diet and by Diamond and Weinmann (7) in a syphilitic fetus. Schour and Smith (8), in a study of fluorosis in rats, described marked changes in the appearance of the ameloblasts and a globular deposition of enamel substance on the surface of the organic enamel matrix. Arnim (9) observed similar hypoplastic lesions in the incisor teeth of rats following extraction of the upper first and second molars.

MATERIAL AND METHOD

The jaws of 105 mice were used in this study.[2] Mixed strains of stock mice were selected in order to obviate the possibility of strain variations in susceptibility to infection. All the animals were sustained on an adequate diet consisting of fox chow, oats and lettuce. Fifty-eight of the mice were inoculated intraperitoneally with the bovine type of tubercle bacilli between the 17th and 50th day of life. The amounts injected varied from 0.1 to 0.7 mgm. per 100 gms. of body weight. Twelve additional animals were inoculated with 0.05 to 0.1 mgm. per cent of the human type of microörganism on the 10th day of life. The entire group of 70 tuberculous mice was sacrificed between 186 and 204 days after inoculation. Twenty-two animals, which served as controls,

[1] This work was done while the author held a Carnegie Fellowship placed at the disposal of the School of Dentistry, University of Pennsylvania. Received for publication, October 13, 1941.

[2] The heads of these animals were made available to the author through the kindness of Dr. Bruno Gerstl of the Department of Pathology, Yale University School of Medicine.

were sacrificed at corresponding age periods. An additional 13 uninoculated mice were confined with their tuberculous parents for a period of 4 weeks following birth. They were then removed to separate cages and sacrificed 4 weeks after isolation.

Immediately after the animals were sacrificed, the mandibles were removed and divided into right and left halves. The maxillae were similarly divided so as to include the incisor and molar teeth. The specimens were then fixed in 10 per cent formalin and histologic sections prepared by a modification of the technique described by Burket (10). Longitudinal serial sections of the mandibles of all 105 mice were cut at 8 micra. Similar sections were prepared of 10 maxillae. Every sixth section was mounted and stained with Delafield's hematoxylin and eosin. Mallory's connective tissue stain and the Foot modification of the Masson stain (11) were used in some instances. Several sections also were stained by Ziehl-Neelsen's carbol fuchsin method.

FINDINGS

Autopsy examination of the 70 mice inoculated with tubercle bacilli disclosed gross evidence of tuberculosis in the viscera of all animals but one. Dental microscopic changes were similarly observed in 69 mice of this group. The animal with no dental changes was not the same one that had no visceral lesions.

No gross or histologic lesions were seen in the viscera or developing teeth of the 13 uninoculated mice which were kept in contact with their tuberculous parents. The appearance of the ameloblasts was identical with that observed in the control group of animals.

Since the dental changes were confined primarily to the ameloblasts and the subjacent surface of organic enamel matrix, the remaining dental and supporting structures will be omitted from the histologic description.

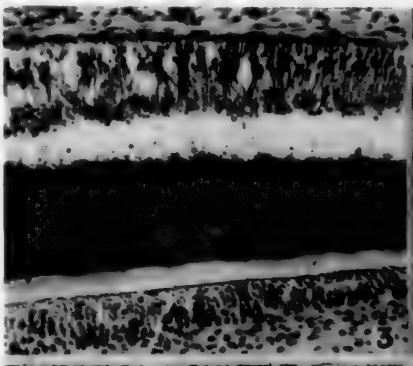
Before reporting the experimental lesions produced in the enamel organ, a brief description will be made of corresponding areas in the incisor teeth of normal mice.[3] The ameloblasts are arranged in a uniform manner (*fig. 1*). They are tall, columnar and their elongated, blue staining nuclei are disposed toward the portion of the cells proximating the stratum intermedium. The cytoplasm is faintly basophilic and granular except at the extreme cell periphery above the nuclei where it is acidophilic. Separating the superior border of the ameloblastic layer from the flattened cells of the stratum intermedium is a distinct cell membrane. The inferior border of the enamel forming cells is limited by a fine basement membrane with regularly spaced, deep blue staining granules situated at the point of junction between the individual ameloblasts. Coursing from the granules toward the surface of the organic enamel matrix are basophilic fibrils which, under high magnification (*fig. 2*), are seen to connect with the individual enamel rods. The outer border of enamel matrix is uniform in appearance and separated from the ameloblastic layer by the zone of vertical fibrils.

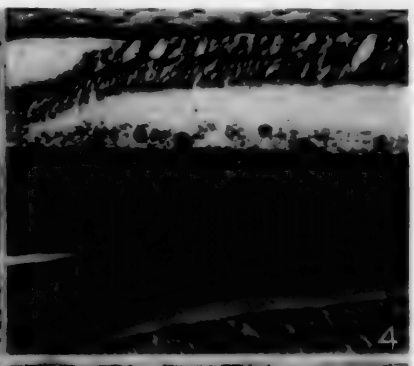
The characteristic changes observed in the tuberculous mice are in sharp

[3] The normal histology of the mouse incisor will be discussed in greater detail in a later report.

FIG. 1. Mandibular incisor of control animal showing normal appearance of ameloblasts and subjacent surface of organic enamel matrix. (orig. mag. ×115)

FIG. 2. High magnification showing normal ameloblasts, subjacent fibrillar zone and organic enamel matrix in greater detail. (Orig. mag. ×280)





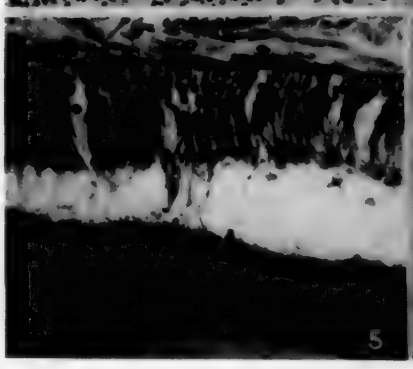


FIG. 3. Mandibular incisor of tuberculous mouse showing irregular and frayed appearance of ameloblasts with pyknosis of their nuclei and enamel-like droplet layer along inferior cell border. (Orig. mag. ×225)

FIG. 4. Ameloblastic injury in tuberculous mouse. Large globules of dark blue staining material are seen overlying outer border of enamel matrix. (Orig. mag. ×220)

FIG. 5. Mandibular incisor of tuberculous mouse. There is vacuolization of ameloblasts with deposition of enamel-like droplets along inferior cell border. (Orig. mag. ×290)

FIG. 6. Irregular deposition of globular material on surface of organic enamel matrix in tuberculous mouse. (Orig. mag. ×200)

contrast to the normal appearance of the ameloblasts and so-called fibrillar zone. The enamel forming cells, in most instances, are markedly fragmented and frayed and their nuclei are pyknotic (*fig. 3*). No basement membrane is seen. Along the inferior border of the ameloblastic layer of cells is a deposit of small, deep blue staining droplets. These are frequently seen as large globules overlying the enamel matrix (*fig. 4*). An interlacing network of faintly basophilic granules and fibrils is present in the same region. The peripheral border of the ameloblasts subjacent to the stratum intermedium is vacuolated in appearance. A high magnification (*fig. 5*) shows, in greater detail, the irregular and vacuolated appearance of the ameloblasts with displacement and pyknosis of their nuclei. To the left is an area of cell destruction with a karyolytic nucleus in the center. Along the inferior cell border is an accumulation of basophilic granules and globules with a tear-drop-like secretion from the vacuolated area in the center of the section. A more striking example of the deposition of globular-like material on the surface of the organic enamel matrix is seen in *fig. 6*. The masses vary greatly in size causing the enamel surface to be irregular in appearance.

Under ordinary conditions of illumination, the globules (in the sections stained with hematoxylin and eosin) appeared similar in their staining qualities to the underlying organic enamel matrix. In those sections where the Mallory's connective tissue stain was used, only the peripheral zone of enamel matrix took the same orange stain as the globules. The remainder of the enamel appeared deep blue. This difference in staining reaction was also demonstrated by the Masson method.

When selective transmission bands were used in studying these sections, it was found by W. M. Hammond[4] that the droplets appeared to be of possibly different chemical structure from the enamel matrix proper. That is, the monochromatic reading showed a difference of tonal values. This observation led Hammond to believe that the drops, if they are of enamel composition, have not reached the same degree of maturity as the main enamel or else that some unusual change has taken place.

In addition to the changes already described, many of the sections show a second layer of tall columnar cells underlying the ameloblasts (*fig. 7*). It is evident from the illustration that this additional layer is in direct communication with the fibrillar network present between the undifferentiated cells of the inner enamel epithelium and the undifferentiated odontoblasts. In the region to the left, where dentin formation has last occurred, the fibrillar zone is greatly increased in width and contains a homogeneous, pale purple staining, edematous-like material. The fibre network is prominent and has a finely granular appearance. Small round, dark staining nuclei are seen in some of the larger fibres which have a tall columnar shape and are arranged more or less uniformly over the newly formed enamel matrix. The overlying ameloblastic layer shows a large area of cell destruction to the right with many malposed, pyknotic nuclei. In some instances, the fibrillar and granular layer extending from the delicate

[4] Personal communication from Mr. Ward M. Hammond, Research Associate in Microscopy, School of Dentistry, University of Pennsylvania.

fibre network between the undifferentiated enamel and dentin forming cell layers, is seen to overlie the enamel matrix surface for a relatively long distance

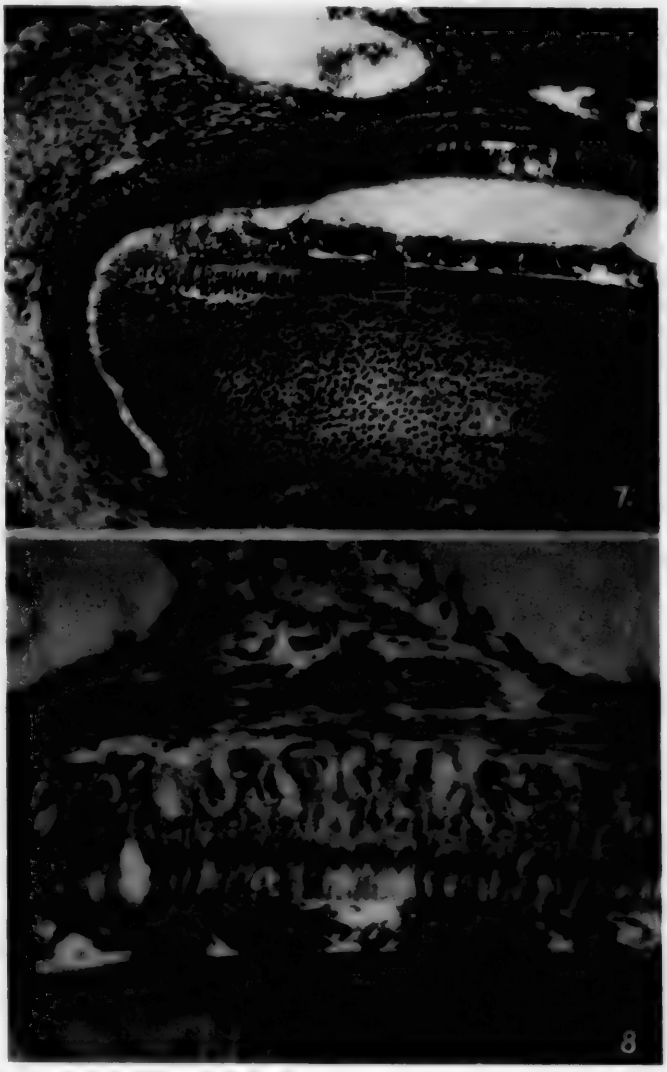


FIG. 7. Mandibular incisor of tuberculous mouse showing layer of tall columnar cells underlying ameloblastic layer. Note communication of this cellular layer with fibrillar zone separating inner enamel epithelium from undifferentiated odontoblasts. (×190)

FIG. 8. High magnification of abnormal cell layer between ameloblasts and organic enamel matrix. Large area of ameloblastic vacuolization is seen to left. (×335)

before any distinct cellular transition occurs. The secondary cell layer finally terminates at some point along the inferior border of the ameloblasts. There is

no protoplasmic continuity of the cells in this region. That the duplicate layer is not a result of artefact is evidenced by its site of origin, the absence of tissue superimposition and the distinct ameloblastic basement membrane.

A higher magnification (*fig. 8*) shows the above changes in greater detail. Between the formed enamel matrix and the ameloblastic layer is a mass of tall slender cells arranged in haphazard fashion and containing pyknotic nuclei. A large cystic area with crowding and displacement of the adjacent cells is seen in the overlying ameloblasts.

DISCUSSION

It is evident from the present study that experimentally produced tuberculosis in mice can result in injury to the enamel forming cells. These changes are similar to those reported by other investigators and attributed to a wide variety of causes. This similarity of pathologic change resulting from different experimental procedures, is further indication of the possibility that the mechanism of enamel hypoplasia may be the same regardless of etiology and that no distinction can be made by histologic examination with the methods so far utilized. It is thus likely that the various disease processes which can result in enamel hypoplasias do so by altering the same essential factors. No acid fast organisms were found in any of the sections stained with Ziehl-Neelsen's carbol fuchsin.

The characteristic changes observed were injury to the ameloblasts and a deposition of enamel-like droplets along the outer border of the organic enamel matrix. In light of various reports regarding the histogenesis of normal enamel, the presence of small droplets alone would be of no significance. Chase (12) mentions the possibility that the basophilic interprismatic substance formed by the Tomes' processes, is a result of the confluence of the enamel globules secreted by the ameloblasts. The prominence and irregularity of the droplet layer in the tuberculous mice and the associated ameloblastic injury is evidence, however, of a definite disturbance of amelogenesis.

When sections, stained by the method of Masson or that of Mallory for connective tissue, are examined under ordinary conditions of illumination, it is seen that the globular formations are similar in their staining properties to the most recently formed enamel matrix. The underlying enamel, which has reached a high degree of maturity, presents an entirely different staining reaction. Since the abnormal deposition or secretion of the droplet layer occurred last, it is not unusual that this substance, if it is of enamel composition, should resemble the least mature enamel matrix in staining reaction. Hammond, in his examination of these sections by selective transmission bands, noted that the monochromatic reading showed a difference in tonal values. This suggested the possibility that the droplets may be of different chemical composition from the main portion of organic enamel matrix.

In a previous study of human teeth (3), lesions were described which permitted the tracing of the pathogenesis of enamel hypoplasia from its earliest manifestations to the stage of clinically demonstrable defects. The changes ob-

served in the present study bear a striking similarity to the earlier of these lesions.

Two possibilities are considered which may account for the relatively slight degree of hypoplastic change. Since none of the experimental mice show evidence of incomplete enamel formation subjacent to the injured ameloblasts or gross defects in the erupted portions of the teeth, it is possible that the animals may have been sacrificed before the end result of cell destruction could manifest itself. However, the period of time between inoculation and death (186 to 204 days) seems ample for such defects to make their appearance. It is likely, therefore, that the degree of cell injury is not severe enough to result in cessation of enamel formation. If the injury is not so severe as to cause complete cell destruction, it is conceivable that the enamel forming cells may return to normal function leaving merely a prominent incremental line to mark the period of abnormal amelogenesis. Since mice are comparatively resistant to experimental tuberculosis (13), it is to be expected that even large doses of tubercle bacilli may cause little or no disturbance. It is of interest to note that there is no correlation, in the presented material, between the degree of ameloblastic injury on the one hand and the dosage or duration of the disease process on the other. Mice receiving small doses of tubercle bacilli show no less severe dental changes than those receiving large doses, and, similarly, animals sacrificed 186 days after inoculation present no significantly different picture than those with a longer disease course.

A striking and unusual change seen in many of the sections, is a layer of tall columnar cells underlying the ameloblastic layer. Arnim (9), in a study of the incisor teeth of rats following extraction of the upper first and second molars, observed a similar change which he described as a second or duplicate layer of ameloblasts.

It is evident (*fig.* 7), that the abnormal layer of cells is in direct communication with the fibrillar zone separating the inner enamel epithelium from the undifferentiated odontoblasts. The absence of ameloblastic proliferation and invagination precludes any such mode of origin of the secondary layer. Whether the cells are similar to the ameloblasts in function as well as morphology and thus formed the underlying organic enamel matrix, is a matter of conjecture.

SUMMARY

A histologic study was made of the incisor teeth of 70 mice with experimentally produced tuberculosis. All but one animal showed marked changes in the ameloblasts and subjacent surface of organic enamel matrix. No dental abnormalities were noted in a group of 22 mice which served as controls.

The characteristic lesions observed, were irregularity and vacuolization of the ameloblasts with pyknosis and karyolysis of their nuclei, and a deposition of enamel-like droplets of varying size subjacent to the injured ameloblastic layer. Also seen, in many instances, was the formation of a layer of tall columnar cells beneath the ameloblasts.

An additional group of 13 uninoculated mice kept in contact with their tu-

berculous parents for a 4 week postnatal period and then isolated for another 4 weeks, failed to show any dental abnormalities.

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BONE HISTOLOGY IN PERIODONTAL DISEASE[1]

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The progressive destruction of alveolar bone and varying degrees of associated gingival changes in periodontal disease present perplexing problems to medicine and dentistry. Analysis of the literature reveals a lack of unanimity regarding the nature of the bone changes associated with the ultimate loss of teeth seen in this condition.

The loss of alveolar bone has been described (1) as a localized process which results from inflammation in the gingival connective tissue. It has also been claimed (2) that the bone loss in periodontoclasia originates as a primary bone atrophy. A compromise concept is presented by those (3, 4, 5, 6) who recognize two major types of periodontoclasia, a "local" type in which the bone loss results from gingival inflammation, and a "systemic" type in which bone changes resulting from systemic disturbances are the primary etiological factors.

To date sufficient histological studies of bone changes to conclusively substantiate these claims have not appeared. It seemed to us that a thorough study of the bone, not only in the periodontal region, but also in the remainder of the jaw would supply valuable evidence for the formulation of an opinion in this matter. In addition, a study of bone from other portions of the body in those cases in which the jaws had been studied would make it possible to determine more definitely whether there were any changes in alveolar bone which did not occur in other bones and might possibly serve as a measure of the extent to which changes in the alveolar bone were the result of local or systemic effects.

This paper records studies designed for the above purposes and specifically considers the following questions: a. Is inflammation an essential prerequisite for alveolar bone destruction? b. What is the nature of the response of alveolar bone to inflammation? c. Are there any changes peculiar to alveolar bone which are pathognomonic of different types of periodontoclasia? d. Is there any relationship between the condition of the bone in the jaws and bone elsewhere in the body?

MATERIAL

In this approach to the problem of periodontal disease, sections from human jaws and other bones obtained at autopsy were studied. Four cases from a series of 17 are presented. These cases are offered because they are from different age groups and present different degrees of periodontoclasia. In each of these 4 cases there is some reduction in the height of the interdental alveolar septa. In Case 1 the amount of bone destroyed is negligible. In Case 2 as much as two-thirds of the root is denuded of bone. The degree of destruction

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in Cases 3 and 4 lies between the extremes of Cases 1 and 2. In every case there is variation in the height of the septa in relation to the individual teeth.

Case 1. (T 239410) Maxilla and mandible. Male, colored, age 39, autopsied 5 days post mortem. For several months before death patient presented ascites with albuminuria. *Autopsy findings:* Thrombosis of portal vein, ascites, splenomegaly, intestinal adhesions and perforation of the cecum with generalized peritonitis. Glomerulo-nephritis was present with no evidence of renal insufficiency. *Oral clinical findings:* Slight marginal gingivitis with no marked ulceration or edema. Slight supragingival calculus on all tooth surfaces with no pronounced gingival recession. Teeth firm, negligible bone loss revealed radiographically.

Case 2. (T 254911) Maxilla, mandible, rib. Emaciated markedly deformed male, white, age 63, autopsied 5 days post mortem. Clinical history of chronic arthritis of long duration. Terminal bronchopneumonia preceded by eight months illness. *Autopsy findings:* Primary scirrhous carcinoma of prostate with metastases to the cervical lymph glands, ribs, scapulae, clavicle and vertebrae. *Oral clinical findings:* Generalized periodontoclasia, pronounced pocket formation, exposure of 4 to 6 mm. of cementum, and loosening of teeth with extensive bone destruction visible radiographically. Marked gingival inflammation, edema and ulceration with heavy calculus deposits on necks of teeth and exposed cementum.

Case 3.[2] (A-40-183 BCH) Maxilla, rib, vertebrae. Emaciated male, colored, age 66, autopsied eight days post mortem. Clinical history of tumor of left lung. *Autopsy findings:* Bronchiogenic carcinoma of the left main bronchus with local extension and metastases to regional lymph nodes, pericardium, diaphragm and right fourth rib. Biliary cirrhosis was an additional anatomical finding. *Oral clinical findings:* Slight gingivitis with edema and no ulceration. Generalized gingival recession exposing approximately 2 mm. of cementum. Teeth firm, slight bone resorption, moderate deposits of calculus and materia alba in gingival crevices.

Case 4. (A-40-7 Camb.) Maxilla, rib vertebra. Female, age 34, autopsied 4 hours post mortem. Clinical history of sub-acute bacterial endocarditis of 6 months duration. Streptococcus viridans isolated from numerous blood cultures. Treatment with sulphapyridine and heparin. Termination occurred with gradually developing unconsciousness and spastic paraplegia. *Autopsy findings:* Sub-acute bacterial endocarditis, evidence of terminal circulatory failure, multiple piarachnoidal hemorrhages. *Oral clinical findings:* Gingivae firm, mild gingivitis with no marked edema or ulceration. Slight gingival recession buccally, lingually and interproximally. Generalized moderate deposits of supragingival calculus. Teeth firm, slight diminution in height of interdental crests visible radiographically.

METHODS

The material was first studied radiographically. Decalcification was then carried out with 5 per cent nitric acid or an equal mixture of 45 per cent formic acid and 20 per cent sodium citrate. With the latter technique there was a minimum of shrinkage and distortion of the cells. Celloidin or paraffin was used for embedding. Greater cellular detail was obtained in paraffin but by decreasing fragmentation celloidin was more effective in maintaining the relationship of the individual tissues.

HISTOLOGICAL FINDINGS

The findings are presented in 3 sections: *Section 1.—Case Analysis—Comparison of the oral clinical condition with the bone findings. Section 2.—Periodontal Findings—Analysis of the bone conditions adjacent to the periodontal mem-*

[2] Courtesy of Dr. Fredrick Parker, Jr., Boston City Hospital.

brane. Section 3.—General Findings—Comparison of the bone conditions in the remainder of the jaw and regions in the skeletal system with those adjacent to the periodontal membrane.

In this study, a discreet layer of pale staining acidophilic homogenous bone matrix, overlying periosteal or endosteal bone surfaces, and covered with one or more layers of somewhat swollen cuboidal or fusiform cells designated as osteoblasts, is recognized as indicative of bone formation. In addition minute foci in which osteoid matrix is laid down in relation to the surfaces of connective tissue cells and fibrils which ultimately become embedded in fully developed bone have also been interpreted as bone formation.

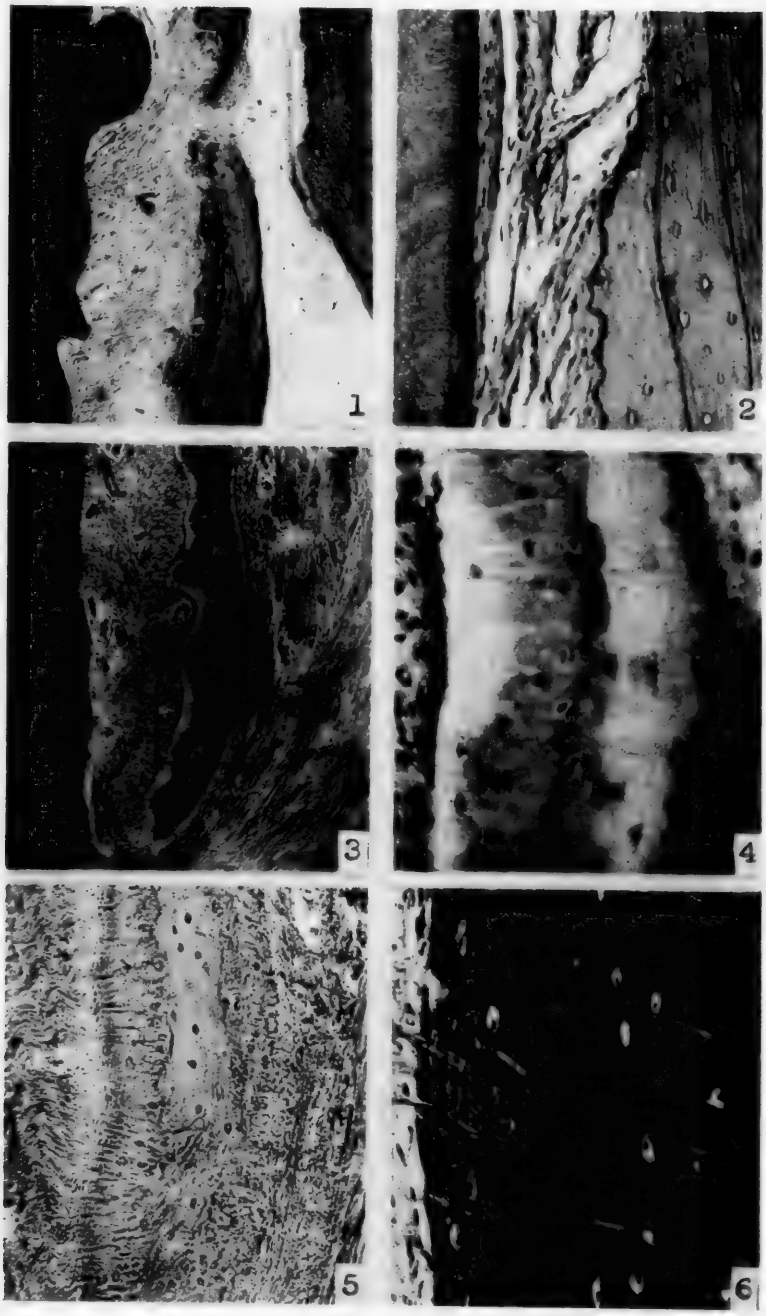
Bone resorption is interpreted as represented by indentations or lacunae which may or may not contain osteoclastic cells and are found along any free bone surface. Such concavities were seen adjacent to the periodontal membrane and along other periosteal and endosteal bone surfaces. Inasmuch as they existed in all cases it is felt that such concavities indicate constant adaptive alterations which free surfaces of bone undergo in response to various stimuli. Predominance of these concavities is considered as indicative of a shift in the bone equilibrium toward the destructive side.

Comparison of the Oral Clinical Condition with the Bone Findings

Case 1. Male, 39, showing only slight gingivitis with a negligible bone loss radiographically. In this patient a tendency toward bone deposition is the striking finding. Adjacent to the periodontal membrane (*figs. 3, 4* and *15*) and labial and palatal periostea (*fig. 23*), lining the vascular channels (*fig. 15*) and along the endosteal margins of bone (*fig. 31*) throughout the maxilla and mandible, a distinct layer of new bone lined with osteoblasts is found. Although there is slight evidence of bone resorption at the gingival margins of the alveolar bone it is overshadowed by the predominance of pale staining new bone laid down on the adjacent trabeculae (*figs. 12, 13*). The histological findings in this case parallel the healthy oral clinical finding and the extremely negligible bone loss visible radiographically.

Case 2. Male, 63, showing pronounced gingival ulceration and extensive bone loss radiographically. In this patient the clinical picture is one of long standing chronic periodontoclasia, with extensive gingival ulceration, heavy deposits of calculus, marked bone loss and loosening of teeth. The histological findings of prominent osteoclastic activity and inflammatory infiltration of vessel channels and marrow spaces are in accord with the clinical picture (*figs. 14, 18, 19*). However bone formation is also found in these zones of inflammation (*figs. 20, 21*). Because of this picture of bone formation and resorption in close association the loss of bone is considered to result from the failure of osteogenic processes to keep pace with the bone resorbing tendencies which may have been stimulated by the chronic inflammation.

Case 3. Male, 66, showing generalized gingival recession with only slight gingivitis and bone loss. In this patient absence of bone formation and generalized bone resorption are the most obvious findings. Along the gingival margins of the interdental crests and adjacent to the periodontal membrane, even in the



FIGS. 1–6

absence of inflammatory involvement, the bone is undergoing metaplasia to connective tissue (*figs. 7, 8*). The vascular channels and endosteal margins in the remainder of the jaw and rib present lacunae of resorption along smooth bone surfaces (*figs. 25, 33, 34, 36*) with no evidence of osteoblastic activity. The degree of destruction of the interdental crests revealed clinically is accurately reflected in the histological picture. However, the generalized distribution of bone resorption in relation to the teeth and throughout the remainder of the jaw, existing in the absence of inflammation, is an occult histological finding for which there is no available clinical index.

Case 4. Female, age 34, showing mild gingivitis with slight bone loss visible radiographically. In this patient bone resorption predominates, with only occasional isolated zones of bone formation. This predominance of bone resorption is seen adjacent to the periodontal membrane (*figs. 2, 9, 10, 11*), along other periosteal surfaces (*figs. 27, 28*), along the vascular channels (*fig. 17*) and lining endosteal surfaces (*figs. 26, 35*). Although the case differed only slightly from Case 1 clinically and radiographically, it presented a markedly contrasting histological bone picture. Whereas in Case 1 bone formation was a striking histological finding, comparable zones in Case 4 presented a predominance of bone resorption. The fact that two cases with similar clinical pictures present diametrically opposed histological findings suggests that there may be present in the periodontal tissues and remainder of the jaw a tendency toward periodontoclasia which has not yet attained sufficient severity or is not of sufficient duration to produce marked clinical changes.

Periodontal Findings—Analysis of the Bone Conditions Adjacent to the Periodontal Membrane

A. *Bone of the Alveolar Sockets.* Irregular hollowed out concavities, containing either multinuclear osteoclastic giant cells or connective tissue elements are

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FIG. 1. Case II. Cementum, periodontal membrane, bone and marrow along mesial aspect of lower canine. Lacunae containing osteoclastic giant cells along bone margin in relation to periodontal membrane. H and E. ×68

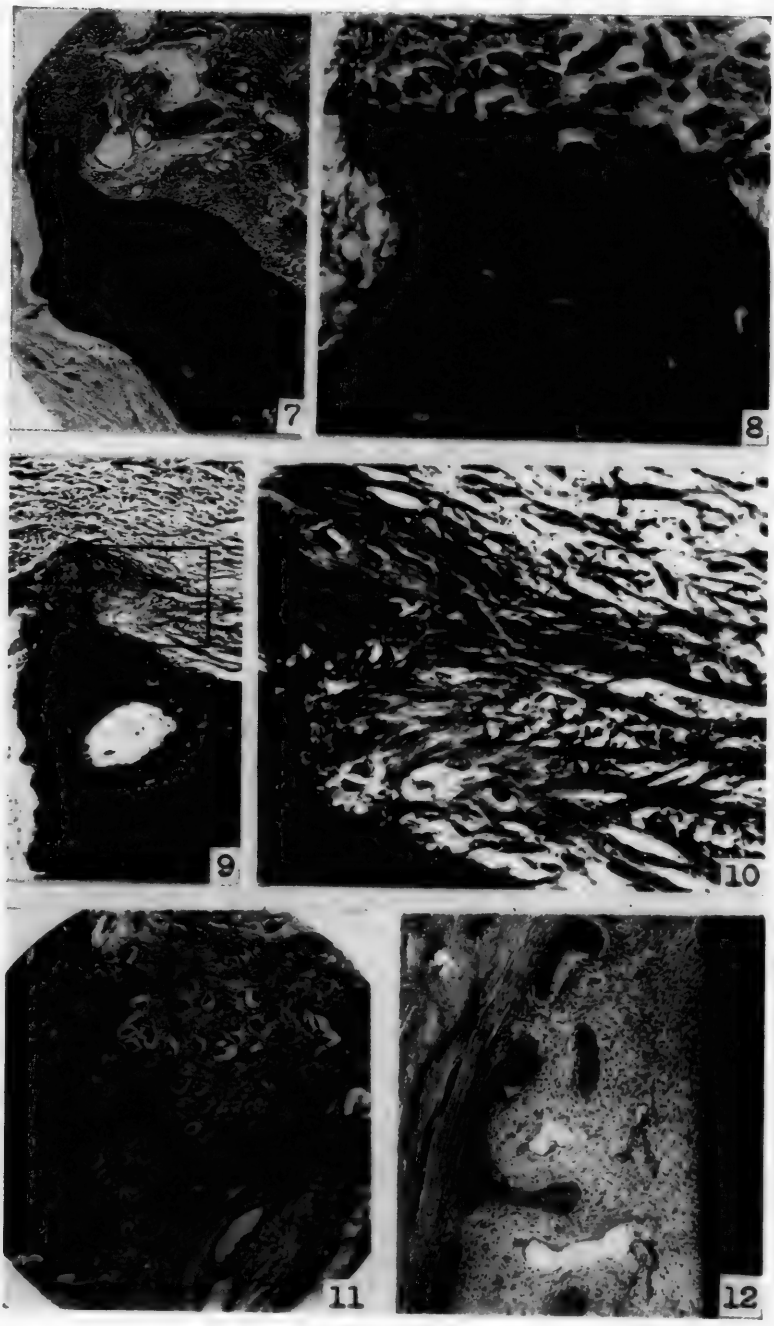
FIG. 2. Case IV. Cementum, periodontal membrane and bone along distal aspect of upper molar root. Concavities containing connective tissue cells and fibres lining margin of bone adjacent to periodontal membrane. H and E. ×162

FIG. 3. Case I. Cementum, periodontal membrane, palatal bone and palatal periosteum of upper canine. Pale-staining acidophilic matrix lining bone in relation to both periodontal membrane and palatal periosteum. Layer of osteoblasts in close apposition with newly formed matrix. H and E. ×152

FIG. 4. Case I. Detail *fig. 3*. Periodontal membrane, layer of osteoblastic cells and bone. Division of bone into 3 zones, newly deposited layer in close apposition with osteoblasts, deeper darker-staining zone and innermost region of adult bone. H and E. ×152

FIG. 5. Case I. Same area as *fig. 3*. Contrast between innermost adult portion of bone and outer zone in relation to periodontal membrane and labial periosteum. Inner zone compactly lamellated, outer zone presents no regular lamellar pattern with prominent Sharpey's fibres continuous with periosteum. No differentiation in degree of calcification of peripheral zones is demonstrated with this stain. VanGieson's. ×148

FIG. 6. Case IV. Bone in relation to periodontal membrane. Extension of Sharpey's fibres for a considerable depth into alveolar bone. Mallory's aniline blue for connective tissue. ×315



FIGS. 7–12

found along the inner margins of the alveoli in all cases (*figs. 1, 2*). These occur independent of inflammation, are not restricted to any particular region of the socket wall, and are more frequent in those cases in which bone resorption predominates in the remainder of the jaw and other bones.

Where active bone formation is taking place adjacent to the periodontal membrane as seen in Case 1, 3 zones can be discerned in the bone (*figs. 3, 4*). Bordering the periodontal membrane is a homogeneous pale staining layer, containing an occasional embedded cell, which is separated from the connective tissue by a layer of flattened spindle shaped osteoblasts lying in close approximation to the bone margin. Further in the septum is a darker staining, apparently more calcified fibrillar layer, in which Sharpey's fibres are markedly prominent. The deepest portion of the septum stains more basophilic than the previous layers and contains regularly arranged compact lamellae. This demarcation of the bone into discreet layers is also seen in relation to the labial periosteum and in bone elsewhere in the jaw as is described with the general findings.

In sections of the above areas stained with vanGieson stain, the innermost compact matrix of bone is sharply demarcated from the 2 peripheral zones described above (*fig. 5*). With this method of staining the 2 peripheral lamellae evident with hematoxylin and eosin staining appear as a single peripheral zone. This demonstrates that the outer homogenous pale staining layer (*fig. 4*) differs from the inner fibrillar layer (*fig. 4*) only in the degree of calcification, and that the fibrillar framework is identical in both (*fig. 5*). The central innermost zone differs from the peripheral laminae not only in degree of calcification but also in degree of organization of the connective tissue framework (*fig. 5*).

Along the inner margins of the alveoli, including the tips of the crests, areas of bone undergoing change to connective tissue are seen, (*figs. 7, 8, 9, 10, 11, 13*).

FIG. 7. Case III. Periodontal membrane and alveolar bone at apex of upper molar root. Bone margin irregular, finely indented and studded with penetrating collagen bundles which are cut obliquely. H and E. ×49

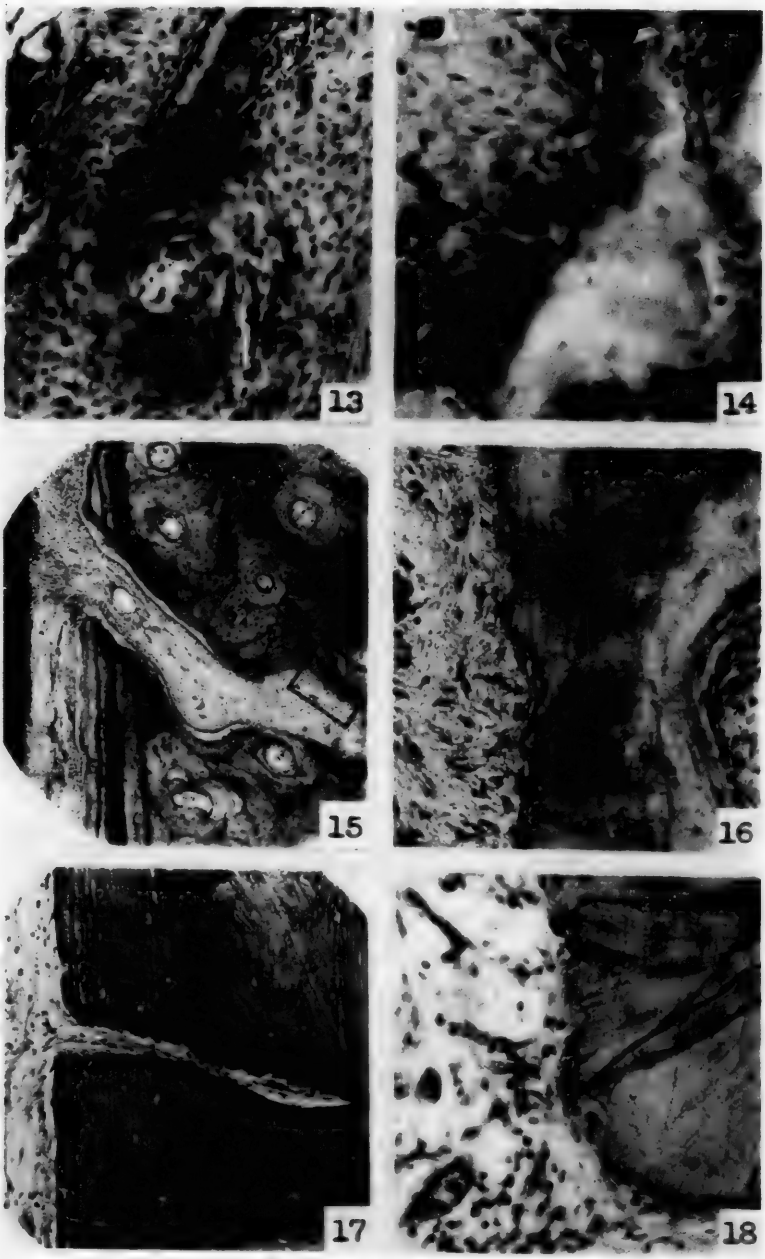
FIG. 8. Case III. Detail *fig. 7*. Bone and periodontal membrane. Collagen bundles and osteocytes in bone. Collagen and connective tissue cells in periodontal membrane. Bone margin rough and granular. Osteocyte at bone margin "released" into connective tissue. Osteocyte nuclei in some lacunae replaced by mucoid ground substance. H and E. ×440

FIG. 9. Case IV. Bone and periodontal membrane at tip of interdental crest, maxilla. Longitudinal sections through collagen fibres in zone in which clear cut demarcation between bone and connective tissue is absent. Mallory's aniline blue for connective tissue. ×114

FIG. 10. Case IV. Detail *fig. 9*. Collagen fibres "denuded" of bone matrix become components of surrounding connective tissue. Outline of lacunae visible in bone matrix. Mallory's aniline blue for connective tissue. ×250

FIG. 11. Case IV. Bone and periodontal membrane. Rough, minutely fragmented, irregular bone margin. "Release" of osteocytes with peripheral dissolution of bone matrix. Cytomorphosis from osteocyte stage to flattened spindle or fusiform connective tissue cell. Mallory's aniline blue for connective tissue. ×169

FIG. 12. Case I. Labial periosteum, gingival margin of labial plate, periodontal membrane and cementum. Upper canine. "Fragments" of bone lying free in periodontal membrane surrounded by connective tissue. H and E. ×62



FIGS. 13–18

These areas are most prominent in Cases 3 and 4. In contrast to the usual appearance of Sharpey's fibres extending for a considerable depth into the adjacent bone from the periodontal membrane, (*fig. 6*) the collagen-fibre-bone-matrix relationship is altered in the areas where the bone is undergoing change to connective tissue. There is a pronounced enlargement of the collagen fibres with an associated expansion in the spaces in which they are embedded, (*figs. 7, 8*) which results in a decrease in bone per unit area and in more closely spaced fibres. In addition, the clear cut demarcation between bone and connective tissue is lost, the bone matrix and periodontal membrane fibres blending imperceptibly (*figs. 9, 10*), and there is peripheral fragmentation of the bone. The matrix assumes a heterogenous granularity which progresses through a fine sand-like stage and ultimately becomes a homogeneous clear background for the surrounding connective tissue. The Sharpey's fibres appear to emerge from the bone shedding the matrix to become fibrillar elements in the connective tissue. In these areas which are undergoing change to connective tissue there is also a progressive increase in the size of the lacunae which become more prominent as the bone matrix is lost and the connective tissue is approached. With fragmentation and dissolution of the matrix the osteocytes undergo cytomorphosis to become dedifferentiated into connective tissue cells (*fig. 11*). All of these changes may occur independently of any inflammatory involvement, (Cases 3 and 4) or may be found associated with inflammation, (Case 2).

B. *Bone in Relation to the Gingival Margin.* In all cases, at the gingival margin of the alveolar sockets, fragments of bone which appear as detached spicules presenting varying degrees of disintegration and resorption, are situated in the surrounding connective tissue (*figs. 12, 13*). These spicules may occur in the absence of inflammation. When associated with chronic inflammation, they

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FIG. 13. Case I. Gingival margin of labial plate, labial periosteum, periodontal membrane. Upper canine. Bone resorption with separation of small bone spicule at tip of plate. Pale-staining new bone matrix lined with osteoblastic cells in apposition with labial periosteum. H and E. ×150

FIG. 14. Case II. Bone fragment in zone of chronic inflammation, periodontal membrane. Multinuclear osteoclastic cells in apposition with irregular bone margin. H and E. ×283

FIG. 15. Case I. Cementum, periodontal membrane, with vascular channel extending into alveolar bone along lateral aspect of root. Well formed Haversian systems. Indented cove-like section of channel margin contrasted with area covered with pale-staining bone matrix lined with osteoblasts. H and E. ×38

FIG. 16. Case I. Detail *fig. 15*. Cove-like indentations along margin of vascular channel filled with connective tissue cells and fibres. New bone matrix lined with osteoblasts along endosteum of neighboring marrow space. H and E. ×132

FIG. 17. Case IV. Lateral aspect of root. Extension of periodontal membrane to form vascular channel. Irregular roughened channel margin with no evidence of osteogenesis. H and E. ×91

FIG. 18. Case II. Resorption lacunae along endosteal surface of bone in relation to marrow containing chronic inflammatory cells. No multinuclear osteoclasts are present. H and E. ×226

appear as small islands of bone lined with multinucleated osteoclastic giant cells (*fig. 14*).

C. *Bone Margins of Vascular Channels.* Extending from the periodontal membrane into the surrounding bone and serving as links between the periodontal membrane and marrow spaces, channels of varying widths containing blood and lymph vessels are present in relation to all the teeth in each case. The margins of these Volkmann's canals present a different appearance in each individual and a variable picture along the individual canal walls. There is considerable variation in the width of the canals which is unrelated to the nature of the changes seen along the margin.

A striking difference is noted between these canals in Case 1 and Cases 3 and 4. In Case 1 the predominant finding along the channel surfaces is a lightly staining homogeneous matrix of varying thickness, the border of which is sharply defined by a layer of osteoblasts which separates it from the surrounding tissue (*fig. 15*). However, some small areas of these same channels present an eroded grooved surface devoid of any new bone formation (*figs. 15, 16*). In Cases 3 and 4 the absence of osteogenesis and the predominance of concavities of resorption represent the condition of the canal margins (*fig. 17*). The concavities contain osteoclasts in some areas and in others are filled with connective tissue cells. The intervening margin of bone separating these concavities is smooth and sharply defined.

D. *Bone Adjacent to Inflammatory Changes Involving the Periodontal Membrane.* Marginal bone changes of varying degrees are found associated with gingival inflammation. In Case 1 there is extremely slight involvement of the gingival bone margins, while in Cases 3 and 4 the inflammatory changes are negligible and limited to the gingival connective tissue. In Case 2 where the inflammatory bone changes are extremely marked, the subepithelial connective tissue stroma of the gingiva is characterized by dense lymphocytic and plasma cell infiltration in addition to proliferation of connective tissue elements. The gingival inflammatory processes progress into the periodontal membrane and by direct extension along the vascular channels extend into the medullary spaces of the bone. With the involvement of the marrow and its displacement by inflammatory elements the resultant microscopic picture is one of mild osteomyelitis. Along the border of the bone approximating the zones of inflammation, in the region of the periodontal membrane and the marrow spaces, are areas of active bone resorption. The bone surfaces present an escalloped appearance created by individual cove-like indentations. In some of these indentations multinuclear osteoclastic giant cells are found in close proximity to the bone (*fig. 19*) while others contain connective tissue and inflammatory cells (*fig. 18*).

The response of the bone in areas involved by inflammation is not limited to resorption; bone formation may also take place. The same bone trabecula which is lined by lacunae of resorption on one aspect may present another surface lined by flattened spindle shaped osteoblastic cells in close apposition to a pale staining homogeneous newly formed layer of bone (*fig. 20*). Similarly, adjacent to zones of inflammatory granulation tissue, the border of the bone may be lined

with a clearly defined margin of pale staining newly formed matrix (*fig. 21*). The occurrence of bone formation and bone resorption in close association is not limited to zones of inflammation. It is a common finding in relation to the periodontal membrane and other areas of the jaw in Cases 1 and 2 (*figs. 13, 14*).

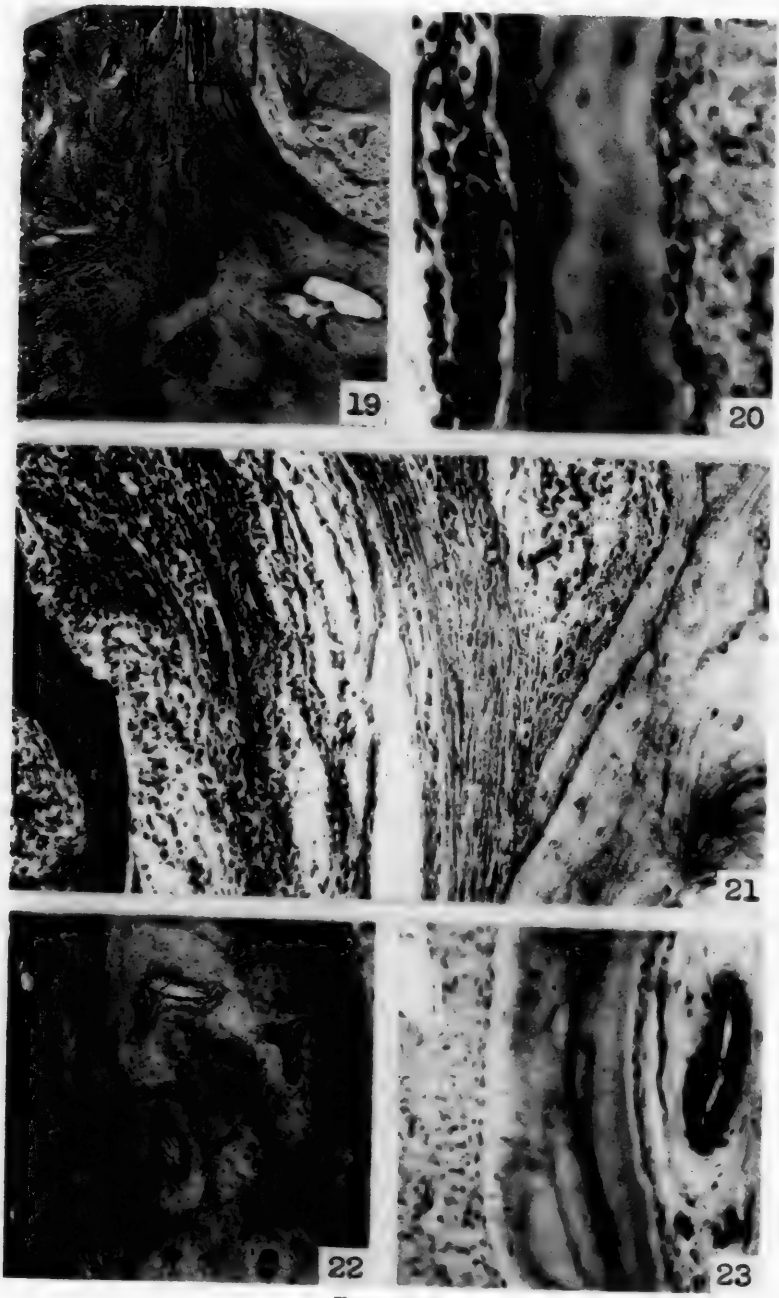
General Findings—Comparison of the Bone Conditions in the Remainder of the Jaw and Regions in the Skeletal System with Those Adjacent to the Periodontal Membrane

A. *Periosteal Bone Surfaces.* The periosteal bone surfaces in regions of the jaw other than those in relation to the teeth and in other bones present varying degrees of bone formation or bone resorption in each case. In Case 1 the periosteal zones show a predominance of bone deposition with slight evidence of bone resorption. The gingival portion of the palatal aspect of the maxilla is covered with swollen osteoblasts beneath which is a thin layer of acidophilic bone matrix. Sharply demarcated from this area, proceeding apically, the remainder of the surface is rough and finely indented with no evidence of inflammatory change (*fig. 22*). The labial aspect of the maxilla presents a similar distribution of osteogenic activity. The zones of bone deposition adjacent to the labial and palatal periostia are similar in appearance to the neighboring periodontal surfaces (*fig. 23*). Penetrating bands of collagen, Sharpey's fibres, are numerous on both labial and palatal aspects of the bone.

In Case 2 bone resorption predominates along the periosteal zones. The inflammation and associated osteoblastic and osteoclastic activity observed adjacent to the periodontal membrane is lacking. At the attachment of muscle to periosteum in the mandible the bone surface is rough and indented. Cement lines and osteocyte lacunae are exposed to the periosteal surface in this area (*fig. 24*). A few small foci of new bone formation are found in the periosteal zones in this case. The bone in the rib of this case was almost entirely replaced and reorganized by metastatic carcinoma and was not considered.

In Cases 3 and 4 the periosteal surfaces of the maxilla are similar to the periodontal surfaces (*figs. 25, 26*). In Case 4 at the posterior margin of the maxillary tuberosity prominent bundles of collagen fibres are seen penetrating the bone. In the same zone the bone matrix fades away and blends with the periosteal tissue in a manner similar to that adjacent to the periodontal membrane (*figs. 9, 10*). The periosteal surfaces of the rib and vertebrae in Case 4 are comparable to the other periosteal and periodontal surfaces in this case in that bone resorption is prominent and bone formation negligible. Many shallow identations roughen the periosteal surfaces of the rib and vertebrae (*fig. 28*).

Broad bands of collagen fibres penetrate the periosteal zones in the rib and vertebrae in a manner similar to the penetration of the interdental septum by fibres of the periodontal membrane (*figs. 29, 30*). Because these penetrating fibres are arranged in bundles, dental investigators (7) have applied the term "bundle bone" to the bone immediately adjacent to the periodontal membrane in which the fibres are imbedded. However, because this term has no significance to histologists and pathologists and because the structure it designates is not a



FIGS. 19–23

special type of bone (*figs. 29, 30*) peculiar to tooth sockets, we feel its use should be discontinued and have therefore not included it in this paper.

B. *Endosteal Bone Surfaces.* In general the appearance of the endosteal surfaces of the bones in each case is similar to the respective periosteal and periodontal surfaces. In Case 1 the predominant picture along the endosteal surfaces is bone formation (*fig. 31*). For the most part the new matrix overlies older basophilic bone and there is a sharp line of demarcation separating the zones. There is no apparent pattern governing the distribution of bone formation and bone resorption. Bone formation predominates but regions of bone resorption are interposed here and there (*fig. 32*).

The endosteal margins of the mandible in Case 2 are finely indented or smooth. There are occasional small foci of new bone formation. No osteoclasts are associated with the small indentations of resorption. In general, the equilibrium of the bone is shifted toward resorption.

In Cases 3 and 4 the endosteal surfaces are similar in the jaws, rib and vertebrae. All show predominant resorption. The bone trabeculae are unusually narrow. The surface resorption is indicated by the presence of small indentations. Very rarely osteoclasts may be found in these indentations in Case 4. Otherwise the endosteal surfaces are smooth and appear quite inactive (*figs. 25, 27, 33, 34, 35, 36*).

C. *Bone Margins of Vascular Channels.* The bone bordering the vascular channels in the ribs, vertebrae, and alveolar bone not adjacent to the periodontal membrane is similar in appearance to that of the bone margins of the alveolar sockets, the periosteal bone margins, and the remaining endosteal margins in the individual cases. A similar condition along the vascular channels adjacent to the periodontal membrane in each case was described in Part I.

In Case 1 the vascular channel margins are predominantly the sites of bone formation although occasionally there are channels which have a finely scalloped margin indicative of resorption. The vascular channels are relatively narrow and compare favorable with the size of the vessels which occupy them (*fig. 22*). The space between bone margins and vessels is occupied by relatively dense con-

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FIG. 19. Case II. Bone, periodontal membrane and marrow spaces. Bone in zone of chronic inflammation in periodontal membrane. Resorption lacunae containing multinuclear giant cells lining the bone margin. H and E. ×58

FIG. 20. Case II. Detail of *fig. 19*. Resorption along margin of bone trabecula in relation to chronic inflammation in periodontal membrane. Layer of osteoblasts lining other bone surface in relation to marrow space. H and E. ×320

FIG. 21. Case II. Bone margin approximating zone of chronic inflammation in periodontal membrane. Flattened spindle-shaped osteoblastic cells lining layer of newly formed bone. H and E. ×184

FIG. 22. Maxilla, Case I. Indentations of resorption roughen the periosteal surface. Haversian systems are prominent. Pale zones represent recently formed bone, the second zone described in text. Deeper zones represent older bone. H and E. ×58

FIG. 23. Maxilla, Case I. Labial alveolar plate, with periodontal membrane to left. Pale zone of new bone matrix, covered with fusiform osteoblasts overlies mature bone. H and E. ×162

FIGS. 24-30

nective tissue. In the other 3 cases the appearance of the margins is that of bone resorption. Small indentations and smooth bone surfaces are characteristic findings in these cases. This is especially well shown in Cases 3 and 4 (*figs. 25, 26*). The vascular channels are large in comparison with the blood vessels, and the intervening stroma is quite loose and has a mucoid appearance. Neither osteoblastic or osteoclastic activity is observed and there is no inflammation.

D. *Bone Matrix and Osteocytes.* In Case 1, where there has been shown to be a predominance of bone formation, the bone matrix of the remainder of the jaw is similar to the bone adjacent to the periodontal membrane and is clearly divisible into 3 zones. There is a narrow endosteal or periosteal zone of acidophilic homogeneous matrix covered with osteoblasts. In a few regions, this zone is absent as previously indicated. A mid-zone, less distinctly acidophilic, contains thin sharp cement lines and small fusiform osteocytes which occupy the entire lacunae. A deep or central zone is definitely basophilic and the osteocyte lacunae are relatively large in relation to the osteocytes (*figs. 22, 32*). In each zone of matrix the bone is homogeneous and fragmentation is not apparent. Along the margins where there is bone resorption the cement lines are less regular than elsewhere and the osteocytes are sometimes exposed to the surface. These zones of resorption vary in staining reaction; they may be either acidophilic or faintly basophilic. The pattern of the bone canaliculi is regular and clearly defined.

The sections of the maxilla in Case 1 are cut in the labio-palatal plane and Haversian systems are clearly apparent (*figs. 15, 22*). In the maxilla of Cases 3 and 4 which are cut in the mesio-distal plane, Haversian systems are present

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FIG. 24. Mandible, Case II. Haversian systems are prominent. Right margin, at insertion of muscle fibres, is rough and fragmented. Cement lines and osteocyte lacunae are exposed on periosteal margin. Endosteal margin to left, is relatively smooth. H and E. ×54

FIG. 25. Maxilla, Case III. Periosteal surface, to right, and endosteal surfaces show small indentations or are smooth. Haversian systems are ill defined, and are cut obliquely. Marrow is mucoid and fatty. H and E. ×76

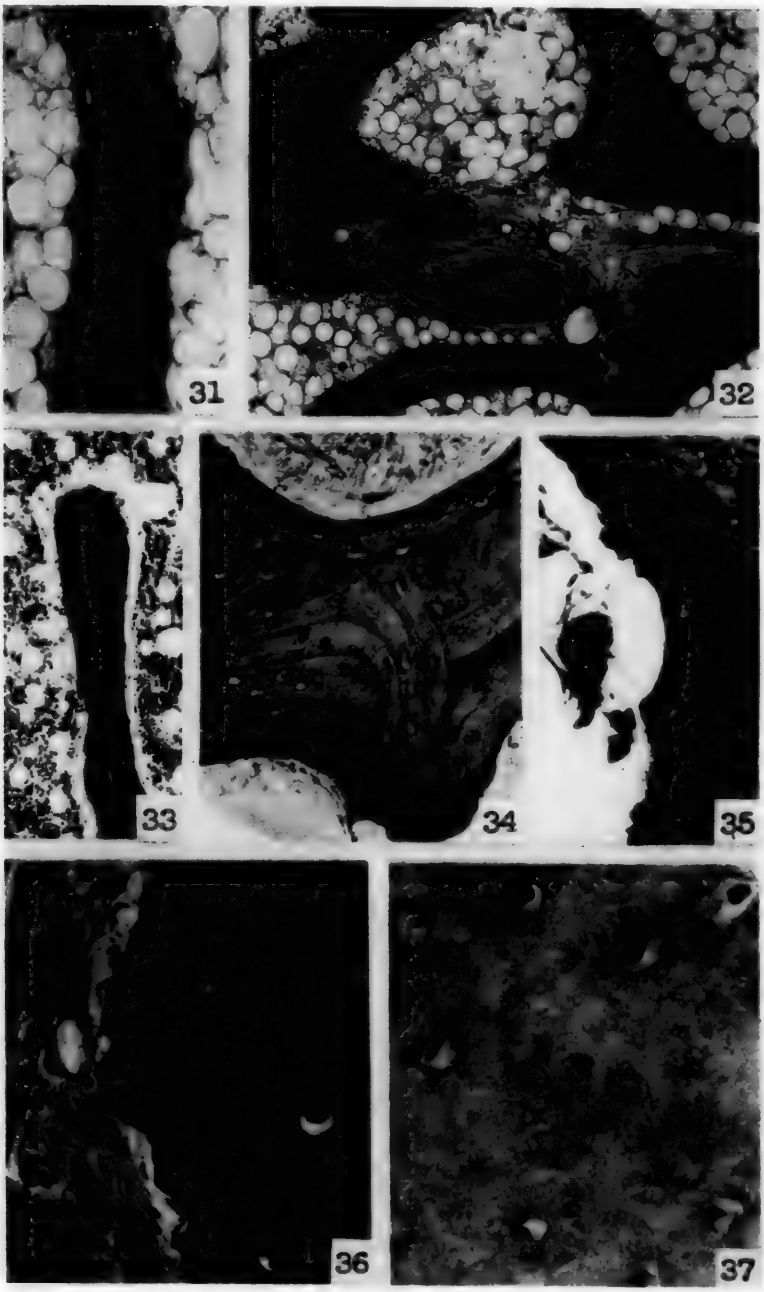
FIG. 26. Maxilla, Case IV. Indentations of periosteal surface, without osteoclasts, are present on right. Margins of vascular channels are rough or smooth. Osteocyte lacunae are large and many contain no osteocytes. H and E. ×152

FIG. 27. Maxilla, Case IV. Bone margin, distal to third molar, is indistinct and finely indented. It is covered with zone of less dense bone matrix, cells and fibres of which emerge with periosteal connective tissue. Collagen bands, Sharpey's fibres, penetrate bone. Marrow is fatty in this field. H and E. ×116

FIG. 28. Rib, Case IV. Periosteal surface, on right, is finely indented and shows no osteoblasts. Lacunae are relatively large. H and E. ×118

FIG. 29. Rib, Case IV. Penetrating bone from periosteum are collagen bands, Sharpey's fibres. Lacunae are large, many contain no cell. Bone matrix is not homogeneous, and tends to be granular. H and E. ×200

FIG. 30. Vertebra, Case IV. Penetration of indented periosteal bone by collagen fibres, cut obliquely, and seen as round or oval foci beneath bone margin. H and E. ×164



FIGS. 31–37

but not well defined (*figs. 25, 26*). The mandible from Case 2, cut mesio-distally, shows prominent Haversian systems (*fig. 24*).

The bone matrix in Cases 2, 3 and 4 differs in appearance from Case 1. The superficial zone of new matrix, so prominent in Case 1, is in general absent in these 3 cases. The smooth or indented bone margins tend to be variable in staining reaction. The matrix is frequently granular and reticulated, and fragmentation is common. There are areas where the matrix shows a transition to periosteal or endosteal connective tissue (*figs. 7, 8, 9, 10, 11, 27*). Many of the cement lines are broad and deeply basophilic, and some are interrupted by surfaces of resorption. Fragmentation along the cement line is common. Osteocytes are frequently exposed where the indentations of resorption have destroyed the lacunar walls (*figs. 2, 16, 17, 18, 34, 36*).

The deep zone of the matrix in Cases 2, 3 and 4 is more regular and homogeneous than the outer zone. The staining reaction in the deep zone tends to be basophilic. There are, however, numerous small foci where the matrix is granular and slightly acidophilic, and where the pattern of the canaliculi and cement lines is obscured by a reticular granularity. In these foci, the osteocyte lacunae are unusually large, may be devoid of cells or may contain osteocyte nuclei which are small and hyperchromic (*figs. 36, 37*). These findings are most striking in the maxilla of Case 3.

E. *Bone Marrow Spaces.* In Case 1 the marrow of the maxilla is composed of well formed fat cells and occasional connective tissue bands. A few minute clusters of hematopoietic cells are present (*fig. 32*). In Cases 2, 3 and 4 the marrow of the jaw bone is composed of atrophic fat cells embedded in mucoid connective tissue. Scattered bands of well-formed collagen support the blood vessels. In Case 2 there is a patch of hematopoietic tissue well inferior to the

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FIG. 31. Maxilla, Case I. Medullary bone trabecula showing pale zones of new bone on either side, covered with osteoblasts. Underlying basophilic indented cement lines suggest previous surfaces of resorption. Central old bone is basophilic. Marrow is fatty. H and E. ×126

FIG. 32. Maxilla, Case I. Medullary trabeculae show 3 zones of bone, but in some regions surface is indented and lacks layer of new matrix. Marrow is fatty. In center left, there is small cluster of hematopoietic cells in connective tissue. H and E. ×58

FIG. 33. Rib, Case III. Medullary trabecula has indented, granular and smooth surfaces. Osteocytic lacunae are large. Marrow is composed of hematopoietic tissue and fat. H and E. ×58

FIG. 34. Maxilla, Case III. Smooth or slightly indented endosteal surfaces of trabeculum. Lacunae are relatively large. Matrix is granular. H and E. ×240

FIG. 35. Maxilla, Case IV. Osteocyte overlying indentation of resorption in absence of inflammation. Bone matrix is granular and large osteocyte lacuna is practically exposed on resorbing margin of bone. H and E. ×440

FIG. 36: Maxilla, Case III. Indented margin of medullary trabecula, with osteocyte practically extruded from bone. Granularity of matrix, with large empty lacunae. H and E. ×440

FIG. 37. Maxilla, Case III. Focus of marked reticularity and granularity in matrix, with large lacunae, some of them empty, showing unusually large canalicular origins. H and E. ×500

first premolar of the mandible. No hematopoietic cells are identified in the maxilla of Case 3. In the maxilla of Case 4 a few hematopoietic cells are identified in the vicinity of blood vessels. The vertebral marrow of Case 3 (*fig. 33*) and Case 4, and the rib marrow of Case 4 are composed of intermingled adipose and active hematopoietic tissue.

DISCUSSION AND SUMMARY

Interpretation of the findings was made in full recognition of the limitation of histological patterns as indices of pathological tendencies. No arbitrary differentiation between a physiological or pathological type of bone formation or bone resorption was made on the basis of morphology. Nevertheless, the histological findings represent tangible indices of the extent of tissue change in the individual cases and as such offer a fundamental basis for the evaluation of studies made in other phases of the periodontal problem.

Our observations revealed no morphological basis for the differentiation of alveolar bone from cancellous bone elsewhere in the body or for the differentiation of the bone adjacent to the periodontal membrane from bone along other periosteal surfaces. All of the bone changes exhibited adjacent to the periodontal membrane could be duplicated along the surfaces of bone trabeculae in the remainder of the jaw and other bones studied. Further, the predominance of bone formation or bone destruction exhibited adjacent to the periodontal membrane in the individual cases could be demonstrated equally as well in the remainder of the jaw and the other bones.

The finding of bone formative and bone resorptive activities in close proximity to each other adjacent to the periodontal membrane and along other bone surfaces points to the fact that periodontoclasia is not simply a process of bone destruction but that it is a condition in which there is a relative predominance of bone resorption over bone formation. Although the bone loss adjacent to the teeth is the most striking clinical finding in periodontoclasia it must be noted that those cases which presented a predominance of bone resorption in the periodontal bone in the absence of inflammation, also presented a predominance of bone resorption in the remainder of the jaw and in such other bones of the body as were studied. These observations suggest first, that periodontal bone loss can occur in the absence of local irritants, and second that periodontal bone loss may represent a manifestation of a generalized tendency toward bone resorption demonstrated in other bones in the individual case.

The loss of bone in periodontoclasia has been shown to occur in different ways. None of the resorptive mechanisms responsible for this bone loss was peculiar to the periodontal bone. All were demonstrated elsewhere in the skeletal system. There is, therefore, no specific type of bone resorption which is peculiar to the periodontium. In an individual case of periodontoclasia, the loss of bone may result from a combination of resorptive processes. It appears, therefore, that periodontoclasia is not a specific type of bone disease and that periodontoclasia cannot be classified into different types on the basis of bone changes.

The finding of bone formation as well as bone resorption in zones of periodon-

tal inflammation is of considerable significance. This finding demonstrates that inflammation in the periodontal area is not the sole determinant of the degree of bone loss in cases of periodontoclasia in which inflammation is present. Both bone formation and bone resorption may occur in the presence of inflammation and the degree of predominance of the latter determines the ultimate bone loss in the individual case. Further, a predominance of bone resorption which is the expression of an intrinsic bone tendency manifested in other bones of the body may exist in the periodontal bone in the absence of inflammation. The effect of inflammation on periodontal bone must therefore be considered as secondary in nature subject to regulation by intrinsic bone tendencies.

The direct association between the periodontal membrane and neighboring marrow spaces by way of vascular channels is of significance. Through these channels pathological changes in the periodontal membrane extend into the marrow and are ultimately reflected in the bone trabeculae in which the marrow is enclosed. Similarly, the vascular channels offer a constantly available avenue through which endogenous disturbances involving the marrow may extend and thereby produce changes in the periodontal membrane.

The presence of bone formation and bone resorption along the same vascular channel points to the extremely flexible morphological character of these structures. It is by means of such mechanisms that realignment and readaptation of these avenues of circulation is made possible in order to suit the requirements of the individual teeth. The fact that the predominance of bone formation or bone resorption displayed in other areas of the jaw was reflected along the margin of the vascular channels suggests that this adaptation by the channels may also be governed by underlying tendencies in no way related to local tooth environment.

The appearance of bone spicules at the gingival margin of the alveolar sockets is not of marked significance. Sectioning technique may be advanced in partial explanation of such microscopic findings. There are, however, zones in which isolated fragments do result at the socket margins because of the peculiar distribution of resorptive processes. A large segment of bone is isolated from the remainder of the bony plate and becomes progressively diminished in size by further resorption, with the eventual formation of a small isolated bone particle completely encompassed by connective tissue.

Comparisons of the clinical oral finding with the histological picture in the individual cases are very informative, particularly because the histological study detects bone formation and bone resorption which cannot be revealed by any known clinical methods. That clinical methods provide a limited understanding of the basic bone changes occurring in any case is emphasized by the contrasting histological findings in 2 cases (1 and 4) which presented similar clinical oral pictures and contrasting histological bone findings. Although there was no difference in the degree of bone loss visible radiographically in these cases, the histological findings revealed a predominance of bone formation throughout the jaw in one case (Case 1) and bone resorption in the other (Case 4). On the basis of this evidence, limited though it is, it cannot be too

strongly emphasized that in the evaluation of the more obvious local and environmental factors in the oral cavity in periodontoclasia it is necessary to consider the clinically undetectable tendencies which are capable of altering the response of the individual's periodontal structures to extrinsic stimuli.

CONCLUSIONS

Inflammation is not an essential prerequisite for the destruction of alveolar bone. Destruction of alveolar bone may occur in the absence of inflammatory involvement. Although bone resorption does occur adjacent to the periodontal membrane associated with the extension of gingival inflammation, bone formation may also be found under the same conditions.

Extensive alveolar bone resorption may occur in the absence of osteoclasts. Osteoclasts are more frequently found associated with inflammation and microscopic sequestra.

The predominant tendency toward bone formation or bone resorption exhibited adjacent to the periodontal membrane is simultaneously paralleled in the remainder of the jaw and other regions in the skeletal system.

Identical bone changes were found in cases representing different age groups and presenting different degrees of periodontoclasia clinically.

Bone destructive and bone formative changes for which there are no clinical indices were revealed histologically.

No special types of bone changes were found which were peculiar to the bone in the vicinity of the teeth or were pathognomonic of periodontoclasia. No special types of bone change were found which justify a classification of periodontoclasia on a histological basis. Therefore, periodontoclasia is not a specific type of bone disease, nor can it be classified into different types on the basis of bone changes.

The authors are indebted to Dr. Howard M. Marjerison and Dr. Charles E. Hatch, formerly Dean and Head of the Department of Clinical Dentistry of Tufts College Dental School respectively, for creating the opportunity and providing the facilities for the initiation of the Project in Periodontoclasia of which this paper is the initial report. We are grateful to Dr. Basil G. Bibby, Dean of Tufts College Dental School, for advice in planning this project and assistance in preparing the manuscript, and to Dr. H. Edward MacMahon, Head of the Department of Pathology and Bacteriology of Tufts College Medical and Dental School, for assistance in preparing the manuscript. We are also indebted to Dr. H. Spencer Glidden of Tufts College Medical and Dental School who, by providing most of the material, makes the continuance of this project possible.

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DENTAL CARIES IN THE PANAMA CANAL ZONE[1]

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In this paper will be reported the results of a dental caries survey[2] carried out on school children of the Panama Canal Zone, showing rather astonishing affects of migration and continued residence there by American children. Much has been written about the low incidence of dental caries among tropical natives living on primitive diets and its marked increase when white flour, salt, sugar, and other processed foods come into common use. Similar caries behavior has been described for primitive people living at all latitudes from equator to pole. No carefully conducted caries survey has yet been reported, however, to show how caries incidence might differ between tropical and temperate zone residents living under similar economic and health conditions but in more civilized states. Nor have any statistics been reported showing what influence migration from a temperate to a tropical climate might have upon caries prevalence.

In view of the unique conditions existing in the Canal Zone, it seemed desirable to compare caries prevalence there with that existing in the temperate zone homeland from which most of the Canal Zone white residents had migrated. The Canal Zone population offers the one large mass of white tropical residents who have migrated from the temperate zone to live in the tropics under general health conditions fully as good as exist in the best temperate zone communities. Efficient sanitation in the Zone and adjacent populated portions of the Republic of Panama keeps the incidence of malaria and other tropical diseases down to insignificant levels, while the even and rather mild tropical climate of the Isthmus promotes a much lower incidence of respiratory illness than is found in the most healthful regions of middle temperate latitudes of North America. Except for fruits, fresh green vegetables, and some tubers, the white Canal Zone residents eat food imported from the United States (except fresh beef, which is mainly native or, at a much higher price, from the United States). Milk is produced in ample amounts at the large Mindi Dairy, from cows which have been imported from the United States and fed on ample amounts of imported foods mixed according to the best scientific formula.

The Canal Zone therefore offers almost an ideal opportunity to study the effects of residence in a tropical climate, without having the direct effects of

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[2] The survey itself was conducted by Dr. Chapin and was made possible by the interested cooperation of Brigadier-General M. C. Stayer, Chief Health Officer of the Panama Canal. The senior author's part in the study was made possible through an invitation from the Gorgas Memorial Laboratory to visit Panama for the purpose of studying the effects of tropical climates upon human health.

climate obscured by a high prevalence of infectious and parasitic diseases so common in unsanitated tropical areas. Malnutrition in the usual sense is obviated by use of highgrade imported foods. Into this protected tropical area has gone a continuous stream of migrants from the United States, with a marked increase in this movement in the recent years of new lock construction and defense force expansion. The Zone population thus offers a chance to study the effects of varying periods of tropical residence, and includes a considerable number of people who have resided in the Zone since birth. Close assay of this population mass for direct and largely uncomplicated effects of tropical climate may give information of great biologic importance.

METHODS AND RESULTS

School populations, where attendance is almost universal, offer the best basis for regional comparison of caries prevalence. The recent survey conducted by the U. S. Public Health Service among the school children of some 28 states (1) provided a valuable yardstick of caries incidence, so we attempted to conduct the Panama survey in an exactly similar manner. Examination was made in a regular dental chair, using probes, mirrors and good natural lighting. Any suspicious cavity that would take the probe and support it in position unheld was recorded as caries. Individual records were kept of the number of carious teeth, number of filled teeth, and the number of missing teeth in each mouth examined. Since the regional aspects of the U. S. Public Health Service survey were worked up only for the 12, 13 and 14 year old school children (2), the present report will deal only with the results obtained on these age groups in the Canal Zone. In addition to the caries record on each child, information was also obtained as to age, sex, race, birthplace, and duration of residence in the Canal Zone.

In Table I are given the data thus obtained on caries incidence in the Canal Zone. Contrary to the prevailing belief in low tropical incidence of caries, the American children born in Panama are seen to show a sharply higher caries incidence than do children recently arrived from the United States. Little change in the caries rate takes place during the first two years' stay of migrants to the Canal Zone, but with longer residence caries tends to become almost as prevalent as in Canal Zone-born children. Native Panamanian children exhibit almost as much caries as do the Americans born there.

Since the Canal Zone population is practically all urban in character, its caries incidence should be compared with that of urban residents of the United States. In the study of Mills (2), there was shown a strong latitude gradient in caries prevalence within the United States, with a steady northward increase in caries rate from about 2.7 (per mouth of 12–14-year-old school boys) in the Gulf States cities to 5.9 in cities of the northernmost states. Since a majority of recent migrants to the Canal Zone were formerly residents of northern states the caries rates here reported for children residing less than one year in the Zone are not significantly different from those of school children of similar ages in their states of origin. Rates for negro school children in the United States always average

TABLE I

Dental caries in the Canal Zone

(12, 13, and 14 year old school children)

	TOTAL NUMBER OF 12-13-14 YEAR OLDS	NUMBER OF PERMANENT TEETH CARIOUS, FILLED OR MISSING (PER MONTH)			
		12 year olds	13 year olds	14 year olds	12-14 year olds combined
Boys					
Panamanian-born Americans	82	6.60±	6.82±	8.47±	7.45 ± 0.29
American-born Americans					
Less than one year in C. Z.	104	5.57±	5.43±	6.39±	5.83 ± 0.21
1.0–1.9 years in C. Z.	54	4.13±	5.21±	6.39±	5.19 ± 0.21
Over 3 " " " "	72	5.28±	7.57±	7.21±	6.88 ± 0.30
Over 6 " " " "	56				7.07 ± 0.37
Native Panamanians	316	6.13±	6.35±	8.17±	7.04 ± 0.16
Girls					
Panamanian-born Americans	92	7.54±	8.54±	8.33±	8.25 ± 0.30
American-born Americans					
Less than one year in C. Z.	85	5.63±	7.24±	6.32±	6.48 ± 0.31
1.0–1.9 years in C. Z.	41	5.50±	5.83±	7.88±	6.59 ± 0.43
Over 3 " " " "	59	7.80±	6.61±	7.72±	7.12 ± 0.33
Over 6 " " " "	41				7.49 ± 0.33
Native Panamanians	343	7.00	8.28	8.02	7.28 ± 0.15

	Defective teeth per mouth
Pan.-born Amer. boys	7.45 ± 0.29
Amer. " " " (0–0.9 yrs. in C. Z.)	5.83 ± 0.21
Difference	1.62 ± 0.36
Odds against chance occurrence: 1 to 415.	
Amer.-born boys (0–0.9 yrs. in C. Z.)	5.83 ± 0.21
" " " (6 + yrs. in C. Z.)	7.07 ± 0.37
Difference	1.24 ± 0.43
Odds against chance occurrence: 1 to 19.	
Pan.-born Amer. girls	8.25 ± 0.30
Amer.-" " " (0–0.9 yrs in C. Z.)	6.48 ± 0.31
Difference	1.77 ± 0.43
Odds against chance occurrence: 1 to 175.	
Amer.-born girls (0–0.9 yrs in C. Z.)	6.48 ± 0.31
" " " (6 + yrs. in C. Z.)	7.49 ± 0.33
Difference	1.01 ± 0.45
Odds against chance occurrence: 1 to 6.	

slightly lower than for the white, just as is seen here in comparing the native Panamanians with the Panamanian-born white children.

Evidently some factor (or factors) in the Canal Zone is predisposing to great caries activity, constituting there a health problem even more severe than is

encountered in the middle temperate regions where caries had always been considered to be at its worst. There exist various possible and probably reasons for this high Canal Zone rate, among which should be listed water supply, dietary inadequacy of vitamins and minerals, and the debilitating effect of the tropical climate itself. Each of these deserves careful consideration.

Total hardness and fluorine content of drinking waters have been shown to be inversely related to caries prevalence in city populations of the United States (2). Caries rates tend to be 30 to 40 per cent higher in children using water from surface sources (rivers and lakes) than in children drinking well water, with a rather clear inverse relationship between total hardness and caries incidence. The entire Canal Zone water supply is taken from Gatun Lake and possesses a total hardness less than 50 parts per million as calcium carbonate. Here resides one possible causative factor in the serious caries situation existing in the Zone. Fluoride addition to drinking water has been shown to diminish or prevent caries in rats fed on caries-producing diets (3), while human populations using drinking water of high fluorine content exhibit low caries rates. Natural drinking waters high in fluorine are always of high total hardness, however, so it is not clear which factor is responsible for the lessened caries prevalence. It would seem wise to consider increasing the fluorine content or total hardness (or both) of the Canal Zone water supply. The situation in the Canal Zone seems comparable to that in the Cincinnati metropolitan district, where the general caries rate for 12–14-year-old school boys is 8.2 while in Norwood (a city of over 30,000 entirely surrounded by and contiguous with Cincinnati) the rate is only 5.4. Cincinnati uses water from the Ohio river with a total hardness of 110 p.p.m. while Norwood uses well water with a hardness of 383 p.p.m.

The second factor possibly responsible in some degree for the high caries rate in the Canal Zone is dietary inadequacy in vitamins and minerals. Heavy tropical rainfall for 10 months of the year tends to leach out of the soil soluble inorganic materials and to result in vegetables with inadequate amounts of calcium and phosphorous. We ourselves undertook no studies along this line, but merely mention this possible basis for the serious caries situation in Canal Zone residents. With regard to vitamin deficiency we do have positive evidence at hand. Requirements for the vitamin B complex (in particular thiamin and pantothenic acid) have recently been shown to be sharply higher per gram of food for animals kept in tropical warmth than for those kept at 65–70°F. (4). Preliminary studies in Panama have indicated a widespread thiamin deficiency to exist there in people living on native foods. Native-grown meats and animal products seem to be much poorer sources of vitamins than do similar temperate zone products. Tropical residents thus face a double handicap—a heightened vitamin requirement but lowered availability for the B fractions. Panamanian residents of the Canal Zone buy mainly native meats, while Americans use native beef and American processed meat products of all kinds. Since caries is worse in Zone-born Americans than in Panamanian children, however, the B deficiency of native meats cannot be a factor of major importance in the caries situation.

The tropical climate itself may play a considerable part by causing widespread

undernutrition and a lowered level of general vitality in the population. Such debility arises from two different phases of climatic effects. One has to do with the above-mentioned increase of vitamin requirement faced by people or animals living in tropical heat, together with a lowered availability of at least the B complex vitamins from animal product sources. The other depends upon the enforced lowering of general tissue combustion level, as greater difficulty of heat loss is encountered by the body. With this lowered combustion level goes slower growth, retarded development, and a lowered resistance to infection. A recent height-weight survey of the Zone school children (5) has shown a progressive growth impairment of American children as their tropical residence extends beyond one year. Work on experimental animals has shown this debilitating effect of heat to persist even when all vitamins are given in entirely adequate amounts and with diets of optimal constitution. It may therefore be that tropical malnutrition, from the heat itself, may be a factor of some importance in the caries picture of the Canal Zone.

SUMMARY

From the data presented it is evident that a serious caries situation exists in in the Canal Zone. Caries rates in the Zone are sharply and significantly higher than in most parts of northern United States where it has usually been considered to be at its worst. It is significantly worse in American children born in the Zone than in those migrating there from the United States.

Among possible causative factors in the situation, drinking water composition should be given first place. Caries rates are highest in those American cities deriving their water supply from river or lake sources, such water being low in both total hardness and fluorine content. The fact that the water supply for the Canal Zone is taken entirely from Gatun Lake may thus be of significance. Only in certain northern cities using river or lake water (Cincinnati, Milwaukee, Portland, Me.) does the caries rate rise as high as is here reported for the Canal Zone.

Discussion is also offered of the possible part vitamin and mineral deficiencies in food may play in the Zone caries situation, and also the possible role of tropical debility from the heat itself.

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ACID PRODUCTION AND TOOTH DECALCIFICATION BY ORAL BACTERIA[1]

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Since the action of acid is the only known mechanism by which human enamel can be destroyed and since the bacterial fermentation of carbohydrates is the only established source of mouth acids, the ability of various microorganisms to produce acids under mouth conditions becomes the first measure of their probable significance in the causation of dental caries. That phase of bacterial activity is, therefore, of fundamental importance in investigations of this disease and deserves more careful consideration than has been given to it in the past.

The most generally accepted indications of the importance of any particular bacterial type in caries are either the final pH attained by it in sugar-containing culture media or its ability to continue to produce acids at acidities below that at which dental tissues are actively decalcified. There are, however, two reasons why the value of these criteria should be questioned. In the first place the conditions of pH under which enamel decalcifies are not definitely established. It is known for instance that some decalcification takes place at acidities near neutrality (1) and which are, therefore, well above the pH reached by most microorganisms. Further, the ability of organisms to multiply at acidities below those at which teeth decalcify rapidly,—variously fixed at pH 4.5 (2), 4.0 (3), and 5.2 (4),—has doubtful significance because the buffering action of the dental tissues themselves will prevent the maintenance of such acidities, and even if a pH below the critical point should be reached, it could not persist in proximity to dental tissues for a long enough period to affect bacterial activity.

In endeavoring to determine the relative importance of bacteria as producers of tooth decalcifying acids in the mouth, several considerations seem more important than those just mentioned. 1. The number of any particular type of microorganism present has a direct bearing on its potential acid-production. This in turn is influenced by the rate at which organisms multiply. Aciduric organisms, for instance, which reproduce slowly would show a relatively smaller increase in numbers in the presence of a suitable substrate than would other types. 2. The rate at which various microorganisms form acid is of fundamental importance because the significance of fermentable foodstuffs in the mouth depends upon their being broken down to acids before they are removed by the self-cleansing mechanisms of the mouth or before they are utilized in

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other ways. Recent evidence (5, 6) indicates that the process of acid formation in the mouth is completed very rapidly. The rate at which any type of organism will form acid will depend upon the numbers that are present and the inherent physiological properties of the particular microorganism. 3. Because it is known that the presence of buffers influences the acid production by microorganisms and because there is a possibility that dental tissues might have some special effects on this phase of bacterial activity, the influence of the presence of enamel and dentin on acid production seems an important factor. 4. The question of whether the amount of acid produced by bacteria is related to the actual amount of decalcification of enamel and dentin has not been settled. Evidence on this point would seem to indicate better than anything else the potential importance of various bacterial types in dental caries.

The following sections of this paper describe experiments designed to give information on these points.

METHODS

Relative numbers of acidogenic organisms. Estimates of the total numbers of viable organisms were made by smearing appropriate dilutions (1:100,000 and 1:1,000,000) of saliva collected by chewing paraffin for a period of 1 to 1½ minutes, on plates of glucose-ascitic-Douglas agar containing 0.5 per cent bromcresol purple. All inoculations were made by streaking the media with sterile glass smearing rods, a method which in previous tests had revealed as many colonies as were shown on poured plates. The percentage of acidogenic organisms, forming concentrations of acid greater than pH 5.2 was determined by counting the numbers of colonies which had completely changed the bromcresol purple indicator. Estimates of the numbers of special streptococcal types were made by inoculating 0.1 cc. of 1:10,000 and 1:100,000 dilutions of the same saliva on oatmeal-bread agar prepared according to directions of Belding and Belding (7). The numbers of lactobacilli were determined on plates of Hadley's tomato-juice agar smeared with 0.1 and 1.0 cc. of whole saliva. Counts were made after periods of 48 and 72 hours. Counts of yeast-like organisms were also made on the Hadley agar plates.

Rate of acid production. In the early experiments representative strains of the organisms appearing on the glucose ascitic agar, oatmeal-bread agar or tomato juice agar plates used in the above procedures were transferred to Douglas broth. After suitable growth periods the resultant cultures were diluted to match suitable barium sulphate standards. One-tenth of a cubic centimeter of these suspensions of streptococci and lactobacilli were then inoculated into 10 cc. of Douglas broth containing 2 per cent maltose, 2 per cent sucrose and 1 per cent glucose. Bromcresol purple was added to indicate acid formation. Similar inoculations were also made into other tubes in which one-half of the Douglas broth had been replaced by centrifuged saliva made sterile by heating to 65° for 10 minutes. After intervals of incubation at 37°C. the tubes were examined to determine whether acid had been formed. After 24 hours the amount of acid formed was measured by titration with N/10 NaOH.

In the later experiments a different procedure was used which made possible

actual measurement of the amount of acid formed after various time intervals together with correlated determinations of the pH and the bacterial population. For each organism tested a series of 50 cc. flasks, each containing 10 cc. of 5 per cent glucose Douglas broth were inoculated with 0.1 cc. of a suitably diluted Douglas broth culture. An uninoculated flask served as a control for each series. At intervals single flasks were removed from the incubator for testing. With the streptococci some tests were made at 4, 6, 8, 10, 12, 16, 20, 24, 36 and 48 hours but in general the 4, 8, 12, 16 and 24 hour tests sufficed. Some lactobacilli were tested at 8 hour intervals but determinations at 24, 48, 72 and 96 hours gave all the essential information. Measurements of acid were made by titrating with N/10 NaOH using phenolphthalein as an indicator. The pH was determined by the use of nitrazine paper followed by spot tests (8, 9) with indicator solutions when the pH was below 5.2. Aliquots removed before titration were used for making counts. These were made by smearing 0.1 cc. of suitable dilutions on Douglas agar or tomato juice agar plates.

Acid production in the presence of tooth substance. One hundred and 200 mg. quantities of autoclaved, pure, powdered human enamel and dentin were added to 50 cc. flasks containing 10 cc. amounts of glucose Douglas broth inoculated with oral streptococci, lactobacilli or leptotrichia. After incubation periods of from 4 hours to 2 weeks, during which the cultures were rotated continuously, bacterial counts, pH determinations, acid titrations, and weighings of the remaining tooth tissues were carried out on the contents of individual flasks. The results obtained from a series of such flasks gave a progressive record of the changes occurring in the presence of enamel or dentin which could be compared with the control series containing no tooth substance.

Decalcifying properties of mouth organisms. The method followed was essentially the gravimetric method used by Volker (10) in comparing the acid solubilities of different tooth substances. The loss of enamel or dentin was determined by the difference in the weights of the samples before and after exposure to cultures for different intervals of time. By selection of tooth substance from suitable sources it was possible to compare the solubilities of enamel and dentin from carious and non-carious teeth, from old and young, permanent and deciduous teeth, and from naturally or artificially fluorosed teeth (11, 12). The same tests as these just described for acid production in the presence of tooth substance served for this study.

FINDINGS

Relative numbers of acidogenic organisms. The total numbers of viable aerobic bacteria as revealed in Douglas glucose agar, the percentage of acidogenic organisms, the numbers of streptococci growing on oatmeal-bread agar and the counts of lactobacilli and yeasts on tomato juice agar for 12 salivas from carious mouths are presented in Table I (Section A). The numbers show wide variations from mouth to mouth both in numbers and relative percentages of different types. The average figures are interesting. They show that in carious mouths lactobacilli make up little more than 1/2000 of the total organisms and less than 1/1300 of the acidogenic aerobic organisms. Yeasts represent only 1/25000 of

the total. Although more than 20 times more numerous than the lactobacilli, the streptococcal types growing on oatmeal bread also seem to be a numerically unimportant group. It seems likely, therefore, that the organisms principally concerned in acid production in the mouth are the acidogenic types (mainly streptococci) which have not been identified in this study.

In Section B of Table I are presented the average figures for a series of 7 studies on saliva from adult patients none of whom showed progressive caries. The variations in numbers of organisms in this series were even wider than those for the carious patients. In view of the extreme variations in both groups, no relationship between the numbers of any particular group of organisms and caries activity can be indicated.

Rate of acid production. The preliminary group of tests showed that streptococci produced acid more rapidly than did the lactobacilli. With the former

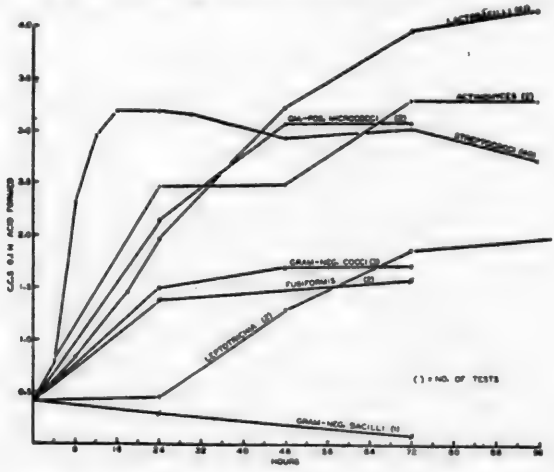

FIG. 1. Rate of acid production by mouth organisms

organisms an acid reaction below pH 6 was reached in from 5 to 10 hours as compared with 9 to 24 hours required by the same sized inocula of lactobacilli. After 24 hours the amount of acid produced by the streptococci was generally less than that formed by the lactobacilli. The averages were 2.6 and 3.2 cc. of N/10 acid, respectively. In the saliva-containing sugar mixtures the average figures were 2.1 for the streptococci and 2.6 for the lactobacilli. Because of technical difficulties satisfactory results were obtained with only 10 strains of each type of organism and these results in themselves differed widely. Therefore, no significance is attributed to these findings.

The method used in the second group of experiments gave consistently satisfactory results, making it possible to establish regular curves representing the rate of acid production for each series of tests. The essential findings are presented in *fig. 1* which compares the average rate of acid production by the different organisms. Streptococci formed acid most rapidly, producing an average

of 2.31 cc. of N/10 acid and a pH as low as 4.5 in 8 hours. Lactobacilli were less rapid in their action, giving an average of only 0.83 cc. acid in the same period of time. In 48 hours, however, they formed an average of 3.23 cc. of acid. Actinomyces ranked second to streptococci in rapidity of acid formation, but like them reached their peak in 24 hours. Gram-negative cocci, Gram-positive micrococci, fusiformis, leptotrichia and a Gram-negative bacillus ranked in that order as acid producers, the last producing absolutely no acid. The average lowest pH given in cultures of these organisms were, lactobacilli, 3.8; streptococci, 4.2; actinomyces, 4.5; Gram-negative cocci, 4.5; micrococci, 4.5; leptotrichia, 5.0; fusiformis, 5.5; and Gram-negative bacillus 7.5. The streptococci fell into two groups relative to acid formation, one forming about 25 per cent more acid than the other (*fig. 2*). In another series the average acid productions by members

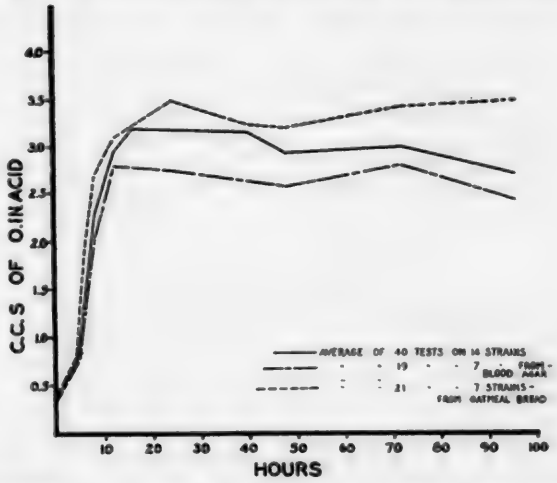

FIG. 2. Average acid production in 40 tests on 14 strains of oral streptococcus

of the two groups of streptococci at 4, 6, 8, 12 and 24 hours were respectively 0.94 and 0.83, 2.13 and 1.51, 2.69 and 2.02, 3.26 and 2.82, and 3.54 and 2.92 cc. of N/10 acid.

Acid production in the presence of tooth substance. Eight series of tests with 4 strains of lactobacilli (*fig. 3*) showed that in the presence of enamel and dentin considerably more acid was formed than when no tooth substance was present, the amount formed with added dentin being greater than with enamel. With leptotrichia (*fig. 4*), however, less titratable acid was found when enamel and dentin were added, the latter showing the greater reduction. Acid production by various streptococcal types was affected differently. Typical results (*fig. 5*) showed that while one strain (OB_2) formed more acid when tooth substance was present another strain (H_8) produced less in its presence. In this chart it is interesting to note the vast increase of acid formation in the presence of rat dentin. Neither decalcified nor ashed rat dentin gave similar results.

While control cultures without tooth substance reached the usual levels of acidity, those containing enamel and dentin never fell below pH 5. The length of time taken to reach this point was related directly to the rate of acid formation being less than 6 hours for some of the streptococci and several days for leptotrichia.

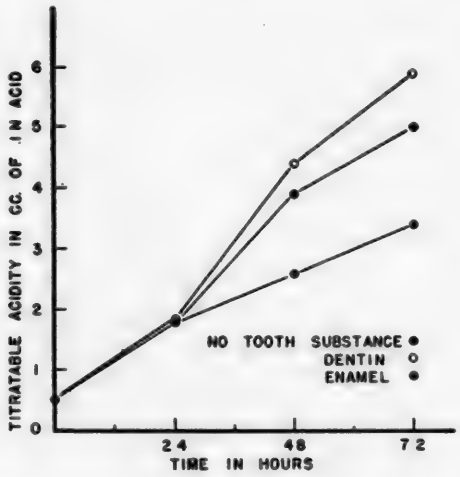

FIG. 3. Average acid production in 8 tests on 4 strains of lactobacilli in the presence of tooth substance

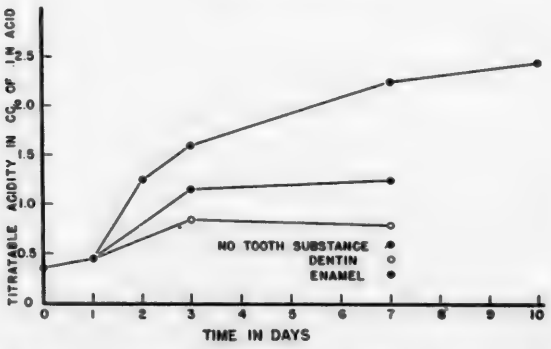

FIG. 4. Average acid production by leptotrichia in the presence of tooth substance

In none of the 18 series of tests on which bacterial counts were carried out was there a significant difference between the rate of growth of any of the streptococci in the enamel or dentin-containing flasks and the control cultures. The only difference noted between the population curves of the OB and H streptococci was that the presence of tooth substance slowed down the death rate of the former (*fig. 6*), whereas that of the latter was not similarly affected and the cultures of the H strains became sterile in 24 hours even when tooth substance

was present (*fig. 7*). These findings indicate that the greater acid production of the OB strains in the presence of enamel and dentin is not the result of augmented bacterial growth. However, the fact that the organisms did not die as

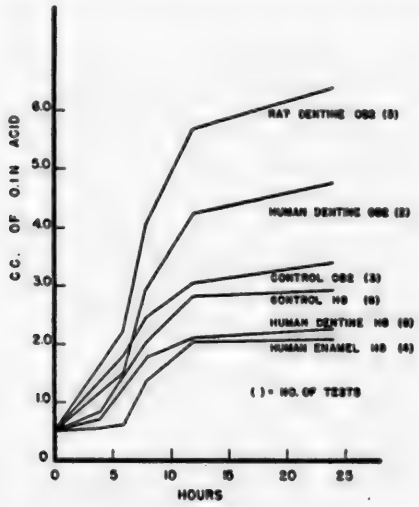

FIG. 5. Acid production by two strains of streptococci in presence of tooth substance

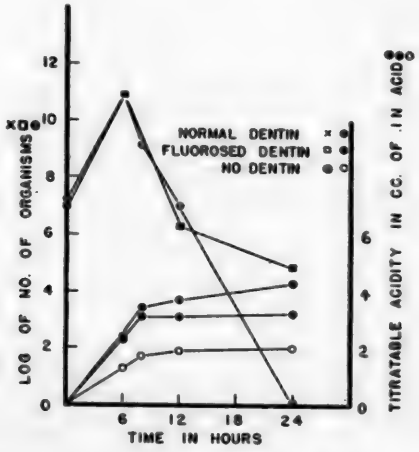

FIG. 6. Bacterial population and acid production in the presence of fluorosed rat dentin

rapidly in tooth substance cultures could account in part for the increased acid formation. This is in accord with the finding with the cultures of the H strains in which there was no increased survival when enamel or dentin were added and no increased acid formation. That something other than the bacterial population influenced acid production was indicated by the absence of any

correlation between the numbers of streptococci, and the amounts of acid formed and by the finding that the acid production and bacterial population curves did

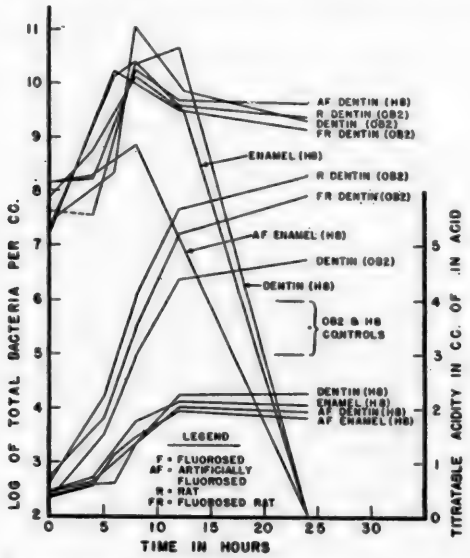

FIG. 7. Average growth rate and acid production in tooth substance experiments

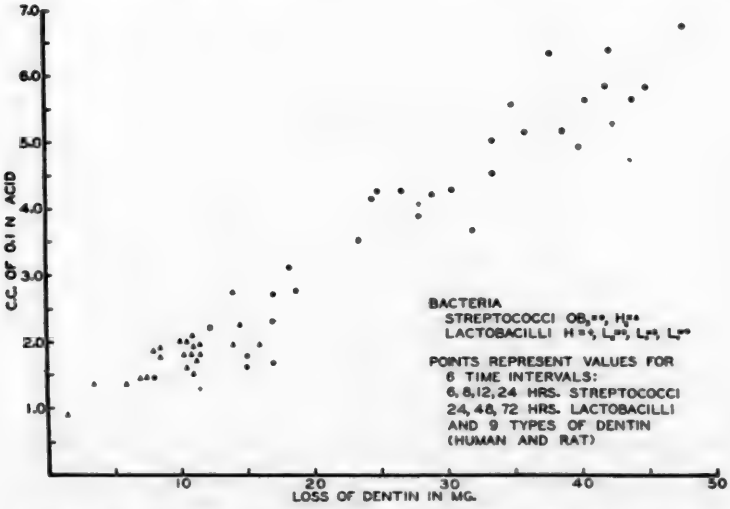
CC. OF .01 N ACID
LOSS OF DENTIN IN MG.
BACTERIA
STREPTOCOCCI OB2**, H**
LACTOBACILLI H**, L2**, L3*, L4**
POINTS REPRESENT VALUES FOR
6 TIME INTERVALS:
6, 8, 12, 24 HRS. STREPTOCOCCI
24, 48, 72 HRS. LACTOBACILLI
AND 9 TYPES OF DENTIN
(HUMAN AND RAT)

FIG. 8. Relationship between the acid production and dentin weight loss

not parallel one another and broke at different times. The relationship between the pH of the media and the acid formation curves seems to cast further light on this matter. With the H streptococci acid production ceased at pH 5,

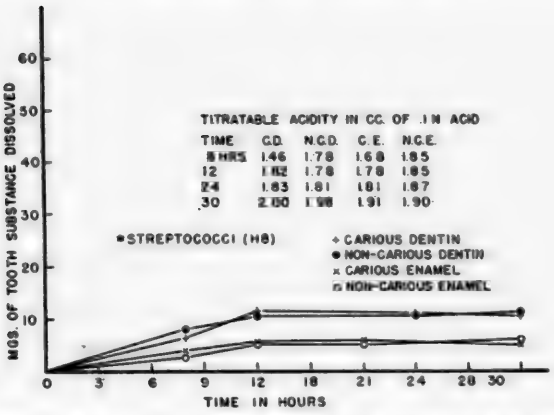
MGS. OF TOOTH SUBSTANCE DISSOLVED
TIME IN HOURS
TITRATABLE ACIDITY IN CC. OF .1 N ACID
TIME C.D. N.C.D. C.E. N.C.E.
8 HRS. 1.46 1.78 1.68 1.85
12 1.62 1.78 1.78 1.85
24 1.83 1.81 1.81 1.87
30 2.00 1.98 1.91 1.90
• STREPTOCOCCI (H8)
+ CARIOUS DENTIN
• NON-CARIOUS DENTIN
× CARIOUS ENAMEL
□ NON-CARIOUS ENAMEL

FIG. 9. Solubility of carious and non-carious tooth substances in bacterial* acids

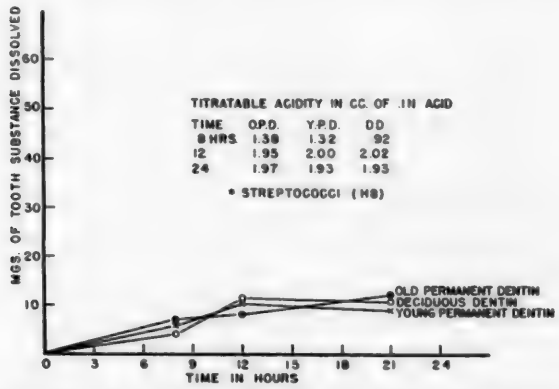

FIG. 10. Solubility of dentin from various types of teeth in bacterial* acids

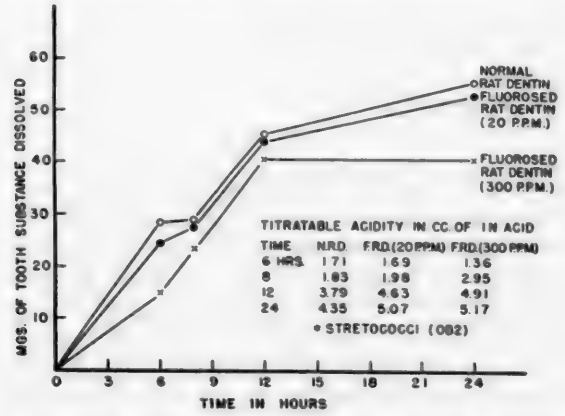
MGS. OF TOOTH SUBSTANCE DISSOLVED
TIME IN HOURS
NORMAL RAT DENTIN
FLUOROSED RAT DENTIN (20 P.P.M.)
FLUOROSED RAT DENTIN (300 P.P.M.)
TITRATABLE ACIDITY IN CC. OF .1N ACID
TIME N.R.D. F.R.D.(20 PPM) F.R.D.(300 PPM)
6 HRS. 1.71 1.69 1.36
8 1.83 1.98 2.95
12 3.79 4.63 4.91
24 4.35 5.07 5.17
• STRETOCOCCI (082)

FIG. 11. Solubility of normal and fluorosed rat dentin in bacterial* acids

whereas with the OB types it continued at or below this acidity. Consequently in cultures of the former acid production and bacterial life ceased at the pH of tooth decalcification whereas with the latter both functions continued.

Decalcifying properties of mouth organisms. Determinations of the amount of enamel and dentin dissolved by bacterial cultures were made only with streptococci and lactobacilli. It was at once apparent that the loss of tooth substance was proportional to the titratable acidity of the cultures. *Fig. 8* shows the straight line relationship which existed for all the dentin determinations. Although the amount of enamel going into solution was much less than in the case of the dentin, a similar straight line relationship held. No differences were found between the solubilities of the various types of dentin or enamel in the bacterial cultures. The amounts of sound and carious enamel and dentin (*fig. 9*) and the amounts of old and young permanent tooth dentin, and deciduous tooth

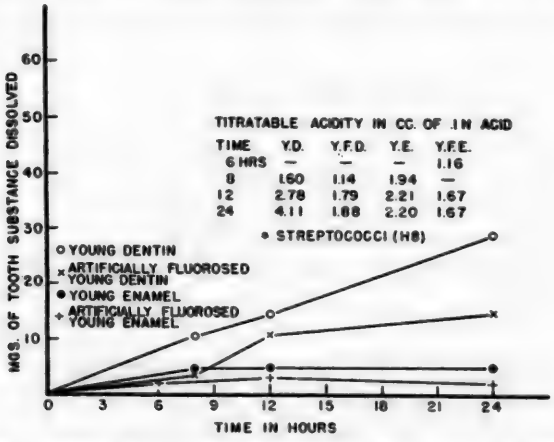

Fig. 12. Solubility of normal and artificially fluorosed tooth substances in bacterial* acids

dentin dissolved were essentially the same (*fig. 10*). Fluorosed rat dentin was less soluble than normal rat dentin (*fig. 11*) and dentin and enamel treated with fluoride solutions ("artificially fluorosed") resisted decalcification better than untreated samples (*fig. 12*).

DISCUSSION

A consideration of the numbers of the different types of organisms cultured from saliva from mouths with active caries (Table I) emphasizes that even under favorable conditions the lactobacilli represent a relatively unimportant minority of the bacteria of the mouth. This conclusion agrees with that drawn from differential bacterial counts made on smears from carious cavities (13). It is particularly interesting to note that the lactobacilli made up less than 0.1 per cent of the strongly acidogenic organisms and that most of these acidogenic types were streptococci. This finding seems to indicate that at the pH ranges

normally existing in the mouth, the streptococci would produce more acid than would the lactobacilli. That the streptococci are an important source of acid in the mouth is made clearer when the rate of acid production is considered. From the data given in *fig. 1* it is seen that they produce acid more rapidly than any of the other bacterial types which were tested, producing in the first eight hours of incubation four times more acid than the lactobacilli. Therefore, since the streptococci are approximately 1,000 times more numerous than the lactobacilli and since it is likely that activity in the mouth parallels that in test tubes, it can be estimated that lactobacilli give rise to no more than .025 per cent of the acid formed by the action of salivary organisms on carbohydrates.

TABLE I

Relative numbers of acidogenic organisms

CASE	AGE	CAVITIES	TOTAL BACTERIA PER CC.	PER CENT ACID FORMERS	STREPTOCOCCI PER CC.	LACTOBACILLI PER CC.	YEAST PER CC.
A—Salivas from the carious mouths of children							
1	16	28	1,300,000,000	76	6,800,000	17,300	1,600
2	16	5	5,000,000	80	2,240,000	51,000	0
3	9	10	1,150,000,000	61	13,700,000	0	26,200
4	12	10	350,000,000	77	1,740,000	400,000	0
5	13	6	26,000,000	61	1,119,000	0	540
6	13	6	16,300,000	70	3,000,000	10,800	500
7	8	8	21,500,000	57	1,820,000	80,000	0
8	13	6	15,300,000	65	720,000	18,200	20,000
9	11	5	23,500,000	77	3,090,000	580,000	46,000
10	8	12	32,000,000	63	2,120,000	220,000	1,200
11	8	6	8,300,000	61	1,500,000	18,000	19,000
12	8	10	24,000,000	73	1,600,000	180,000	4,800
Avg.....	11.3	9.3	247,600,000	68	3,287,000	131,275	9,986
B—Saliva from the non-carious mouths of adults							
Avg. of 7 cases	30	1.5	1,130,000	71	21,730,000	3,270	70

The discovery that some types of organisms give rise to more and others to less acid in the presence of enamel and dentin, suggests that certain bacterial types are especially well adapted to produce acid in the presence of tooth substance. That such differences appear between various types of streptococci is particularly interesting and may be taken to indicate that certain streptococci are particularly important in producing decalcification of the teeth.

The observation that as long as tooth substance was present in the bacterial cultures the pH did not fall below pH 5 is significant. This more than anything else seems to indicate the pH at which enamel and dentin pass readily into solution and the pH at which they exert their maximum neutralizing power against bacterial acids. The direct relationship between the acid formed and

the amount of enamel or dentin loss indicates that this loss is entirely the result of the action of the bacterial acids. This finds support in Volker's demonstration (10) that comparable dental tissues show similar solubility effects when treated with organic acids of non-bacterial origin. The greater solubility of dentin than enamel may have some significance in caries and show itself in the more rapid destruction of the former tissue. The extreme solubility of rat dentin, which is destroyed very rapidly in the mouth, lends weight to this idea. On the other hand, the possibility that there is some specific factor in rat dentin which stimulates acid production, cannot be ruled out. It seems more likely, however, that with rat dentin, and to a less extent with human dentin, their greater solubility as compared with enamel makes them more efficient buffering agents and, therefore, the pH of the cultures are less readily depressed to the points where enzyme action is slowed down.

SUMMARY

Cultural estimates of the numbers of acidogenic organisms in saliva from mouths with active caries, indicated that lactobacilli were a minor constituent of the oral flora.

Comparisons of the rate of acid formation by various mouth organisms showed that streptococci and actinomyces-like organisms formed acid most rapidly, and that lactobacilli and other organisms were less active in this respect. Some streptococcal strains formed acid more rapidly than others.

The addition of enamel or dentin to cultures of oral bacteria influenced differently the amounts of acid formed, apparently depending upon the pH at which the bacterial enzymes act. The streptococci fell into two groups, one forming more and the other less acid in the presence of tooth substance. The pH of cultures containing enamel or dentin never fell below pH 5, indicating that this is the critical point for tooth decalcification.

The amount of tooth tissues dissolved in cultures was directly proportional to the titratable acidity. The solubilities of various types of enamel and dentin in bacterial cultures paralleled those observed in acid solutions.

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RELATION BETWEEN THE INCIDENCE OF DENTAL CARIES AND THE pH OF NORMAL RESTING SALIVA[1]

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The acidity of the saliva is one factor that frequently has been considered and investigated in the study of the etiology of dental caries. Considerable study has been made of the relationship between salivary acidity and the incidence of dental caries, but quantitative and statistical methods for the determination of the coefficient of correlation between these factors have not been generally employed in an effort to establish or disprove such a relationship.

The data for the present study were obtained from the School of Dentistry and the Physiological Chemistry Department of the University of Pennsylvania. Dental records of 351 of the freshman dental students were used to determine the incidence of dental caries in this investigation. All students were males in the age group, 19 to 25 years of age. Approximately 1 month after the dental examinations, the pH of the normal resting saliva of these students was determined by themselves as a laboratory problem. By the use of statistical methods the coefficient of correlation between the incidence of dental caries and salivary pH in the 351 cases was determined from these records.

REVIEW OF LITERATURE

With a few isolated exceptions, determination of the hydrogen ion concentration or the pH of the saliva was not done prior to 1920. Before that time, salivary acidity was measured by titration methods. Among these early studies may be mentioned Chittenden and Ely (1) in 1883, Chittenden and Smith (2), 1885, Szabo (3) and Cohn (4) in 1900. These early investigators did not study any relationship between salivary acidity and dental caries. In 1911 Lothrop and Gies (5), after examination of the saliva of several patients with "extensive decay" and of patients "relatively immune" to dental caries concluded that "no significant relationship between the acidity of the average specimen of saliva to phenolphthalein and the condition of the teeth is shown by these results."

Marshall (6) and Hopewell-Smith (7), 1915, after extensive titration studies reached similar conclusions and could find no significant relation between salivary acidity and dental caries. Bühler and Heer (8) in 1917 stated that the acidity of the saliva, as measured by titration, is reduced in dental caries, but presented little evidence or data to prove that point. D'Alise (9) in 1921 stated that

[1] Abstracted from a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Dental Surgery, from the Thomas W. Evans Museum and Dental Institute, School of Dentistry, University of Pennsylvania. (Received for publication September 5, 1941.)

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there appeared to be no relation between dental caries and the reaction of the saliva. However, the titration studies measured the buffering action of the saliva and did not measure the true acidity of the saliva, which is the hydrogen ion concentration.

The first determination of the hydrogen ion concentration of the saliva appears to have been made by Foa (10) in 1905 by the use of electrometric methods. More extensive electrometric determinations were conducted by Michaelis and Pechstein (11) and Kirk, Crowell and Appleton (12) in 1914. Since these early studies, investigators have used 3 general methods for the determination of the salivary pH: some form of electrometric method, an indicator paper method, or a colorimetric method. With the discovery of the sulphonaphthalein dyes, colorimetric methods became almost universally adopted after 1920.

The possible relation between the pH of the saliva and the incidence of dental caries has been studied by numerous investigators. The first extensive study seems to have been made by Bunzell (13) in 1923 on 274 children and adults. Bunzell concluded that there is no evident relation between dental caries and salivary pH but did not do a statistical analysis to prove his conclusion. In addition, Bunzell failed to mention whether or not he collected his salivary samples under oil. Starr (14) has shown that failure to collect specimens under oil may produce serious error in the pH determinations due to loss of carbon dioxide. McIntosh, James and Lazarus-Barlow (15), by the use of the indicator paper method measured the pH of the saliva of 12 children "free from caries" and 31 children "with caries". They concluded that salivary pH seemed to be inversely proportional to the incidence of caries, but their conclusion appears to be unwarranted by the data presented and the few cases studied. Roskin (16), studying a group of 17 children with "few caries" and 13 children with "active caries" found no significant differences between the two groups. McKeag (17), investigating 80 cases could find no significant differences in salivary pH between a group of 46 boys with "little decay" and a group of 34 boys with "considerable decay". Stern (18), after considering 100 cases, stated that nothing conclusive could be deduced from his data. Karshan, Krasnow, and Krejci (19) found the average salivary pH to be 7.03 in 17 cases that were immune to caries, compared to an average pH of 6.91 for 21 cases with active caries present. An average pH of 7.08 was found in 6 cases of arrested caries. On the basis of these results, they doubted if a relation existed between dental caries and salivary pH. Hubbell (20) found the average pH of 16 caries-free children to be 7.3 and the average pH of 16 caries-susceptible children to be 7.2.

Brawley (21) reported that he found no correlation between quantitative caries indices and pH indices of the resting saliva of 281 children. The indices were established at the beginning and the end of a 12 month period, but when an increase in the caries index at the end of 12 months was compared with the pH of the salivas,

"a definite relationship expressed itself as (a) low pH ranges, high caries indices, (b) high pH ranges, low caries indices."

Brawley's report does not present his data nor his method of analysis. Hanke (22) grouped 48 patients whom he studied, into 3 categories: immune to caries,

not susceptible to caries, and susceptible to caries. He concluded that the saliva of immune persons, with one exception, is characterized by being neutral or slightly alkaline at all times; that the saliva of susceptibles is usually acid in reaction (average pH 6.6); and that

"one is immune to dental caries partly because of the excretion at all times of a saliva that is alkaline and highly buffered."

Ziskin and Hotelling (23) studied the relation between salivary pH and the incidence of dental caries in 324 pregnant and 31 non-pregnant women. Their calculation of a coefficient of correlation showed that in their studies there was no relation between the incidence of dental caries and salivary pH. Uekuni (24) without presenting data, figures, or methods of analysis, concluded that there did not seem to be any relation between dental caries and salivary pH.

With the exceptions of Brawley (21) and Ziskin and Hotelling (23), a quantitative caries index for the determination of the incidence of dental caries in saliva correlation studies has not been previously employed. Likewise, with the exceptions of these two studies, the determination of the coefficient of correlation between a quantitative caries index and salivary pH has not been done. The usual method for the determination of the incidence of dental caries in previous investigations has been to classify patients as "caries-free", "caries-susceptible", "arrested caries", or similar designations. Such a classification is not a standard one and will vary from observer to observer. Also, such a classification does not quantitatively measure variations in the incidence of dental caries.

METHODS

To quantitatively measure the incidence of dental caries for the 351 cases studied, the Bodecker modified caries index (25) has been employed in the present study. The caries indices were computed from the dental records of 351 freshman dental students.

The determinations of the pH of the normal resting saliva were made by the students in the physiological chemistry laboratory under the direction of Dr. Florian Cajori. Krasnow (26) has indicated that the time of day, the metabolic state of the individual, chewing, CO_2 escape from the saliva during collection, the time elapsing between the collections of the saliva and the determination of the pH may all cause significant variations in salivary pH. Grossman and Brickman (27) have demonstrated diurnal variations in salivary pH but both these authors and Popper and Haffner (28) have shown the salivary pH to be fairly constant over long periods of time. In order to reduce to a minimum those extraneous factors which may cause changes in pH, the colorimetric method outlined by Starr (14) had been closely followed. All determinations were done at about 9:30 A.M. To obtain a resting (i.e. unstimulated) saliva, the students were requested not to talk, not to smoke, and not to stimulate salivary flow by mechanical means for 10 minutes prior to the collection of the sample. As a check on the accurracy of the method as done by the students, 4 samples of unknown salivas were distributed to 23 students for independent determinations. The results of these determinations are shown in Table I.

The low standard deviation values 0.13, 0.08, 0.10, and 0.08 of the 4 samples

showed low individual error in the pH readings. As a further check, 94 redeterminations were repeated 2 days later under similar conditions. Table II shows the differences in pH readngs by the same students made on different days.

The means of both series of investigations were pH 6.69 and pH 6.72, respectively. Note that 69.1 per cent of the cases showed a discrepancy of 0.1 pH unit or less and that 87.2 per cent showed a discrepancy of 0.2 pH unit or less.

TABLE I

Standard deviations of pH measurements on standard samples by different students

SAMPLE	pH READINGS	MEAN	STANDARD DEVIATION
A	7.1, 7.1, 6.8, 7.2, 7.2, 7.2, 7.1	7.10	0.13
B	7.3, 7.3, 7.6, 7.4, 7.4, 7.4	7.40	0.08
C	7.3, 7.1, 7.2, 7.2, 7.1, 7.4, 7.3	7.23	0.10
D	7.2, 7.1, 7.0	7.10	0.08

TABLE II

Difference in pH readings on 2 successive determinations

pH DIFFERENCE	FREQUENCY	PER CENT OF TOTAL
0.0	34	36.2
0.1	31	32.9
0.2	17	18.1
0.3	9	9.6
0.4	1	1.1
0.5	2	2.1
Total.....	94	100.0

To determine if both series of pH determinations were significantly different or similar, the following ratio recommended by Mainland (29) was employed:

$$\frac{M_2 - M_1}{\pm\sqrt{\sigma_2^2 + \sigma_1^2}}$$

Where M = mean of each series of determinations; $\sqrt{\sigma_2^2 + \sigma^2}$ = standard error of the difference. A value of 2 or less for the above ratio would indicate no significant difference between the 2 series. The actual calculated value for this ratio was 0.14 which indcated no statistically significant difference in successive pH determinations. Thus, the low standard deviation values of pH determinations on standard samples by different students as shown in Table I and the low value for the ratio

$$\frac{M_2 - M_1}{\pm\sqrt{\sigma_2^2 + \sigma_1^2}}$$

above, show that the pH determinations with Starr's method by the students are reliable for the purpose in hand.

The pH readings ranged from 6.0 to 7.4 and the caries indices ranged from 0 to 58 in the present study. To determine whether the cases under consideration comprised a representative and random sampling of the population, the individ-

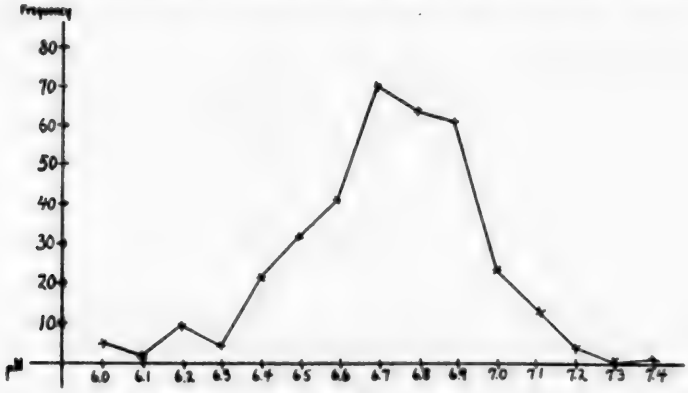

FIG. 1. Distribution of pH readings

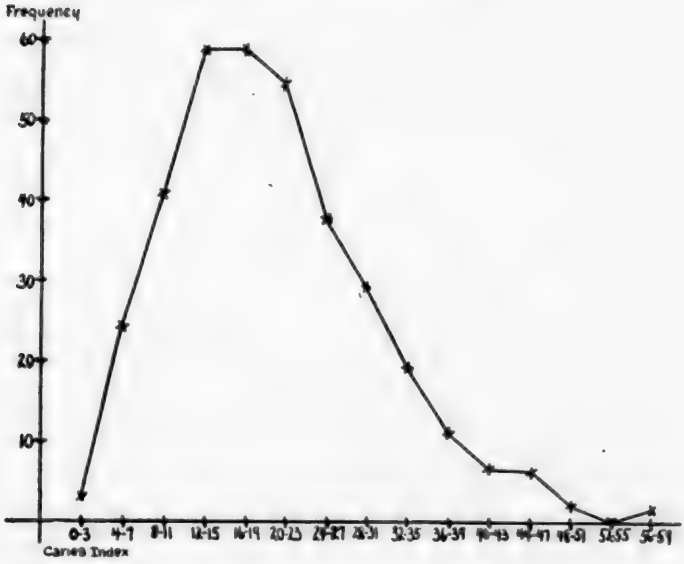

FIG. 2. Distribution of caries indices

ual pH readings and their frequencies were plotted (*fig.* 1) and the distribution of the caries indices with their frequencies were plotted (*fig.* 2).

In both cases, the graphs strongly resembled normal distribution curves which indicated random and representative sampling of the population in the cases under consideration.

Computation of the coefficient of correlation between the pH of the normal resting saliva and the incidence of dental caries was performed by the following formulae given by Arkin and Colton (30):

$$r = \frac{p}{\sigma_x \sigma_y}$$

$$p = \frac{\Sigma(XY)}{N} - \left(\frac{\Sigma(X)}{N}\right)\left(\frac{\Sigma(Y)}{N}\right)$$

$$\sigma_x = \sqrt{\frac{\Sigma(X^2)}{N} - \left(\frac{\Sigma X}{N}\right)^2}$$

$$\sigma_y = \sqrt{\frac{\Sigma(Y^2)}{N} - \left(\frac{\Sigma Y}{N}\right)^2}$$

Where r = coefficient of correlation, σ_x, σ_y = standard deviations, p = a measure of the mean product of the deviations, X = pH readings, Y = Caries Indices, f = frequency, Σ = summation of, N = number of cases.

Calculation of r showed a value of $r = -0.06$. According to Arkin and Colton (30) the coefficient of correlation is used as a comparative measure of association between two series. The significance of values of r are shown in *fig.* 3.

However, even though there was no relation for individual cases, there still remained the possibility that there might be a significant change in the frequency distributions of the caries indices as the pH changed. This might indicate a greater probability of higher caries indices with lower pH readings and vice versa. To test this hypothesis, the caries indices and their frequencies at different pH levels were plotted as shown in *fig.* 4. The extreme pH ranges were omitted due to insufficient number of cases in these ranges.

Note that in each case, there is a peak to the shape of the curve. This permits the assumption that the arithmetic mean of the caries indices may be taken as representative of the whole distribution.

The coefficient of correlation between the arithmetic means of the caries indices at pH ranges 6.4 to 7.0 and the pH readings was determined as previously. The value obtained for this coefficient of correlation was minus 0.44. Upon testing this value with Fisher's (31) test of "t", it was shown that this value was not significantly different from O, which would indicate no correlation. Therefore, we may conclude that there was no evident correlation between the pH of the normal resting saliva and the means of the caries indices corresponding to each pH reading.

Although it has been shown that there is no apparent relationship between the incidence of dental caries and the pH of mixed normal resting saliva, the possibility that local sites of high acidity in the oral cavity may play an important etiologic role in the production of dental caries has not been refuted. We look forward to more study in the future on that phase of the salivary problem. Brawley's (21) suggestion that there is a correlation between salivary pH and changes in the caries indices should be further investigated by statistical studies. More data on the incidence of dental caries in the extreme pH ranges of salivary

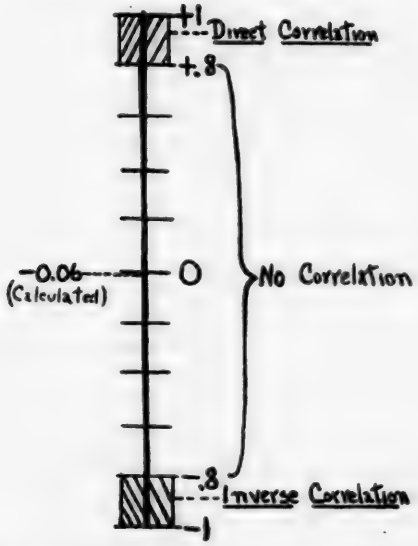

FIG. 3. Significance of "r". Values of r between +1.0 and +0.8 indicate a direct correlation between 2 series; values of r between −0.8 and −1.0 indicate an inverse correlation; and values between −0.8 and 0.8 indicate no correlation, the closer the value to 0, the greater the evidence for no correlation. The value of −0.06 showed at once that there was no evident relationship between the incidence of dental caries and the pH of the normal resting saliva in the 351 cases studied.

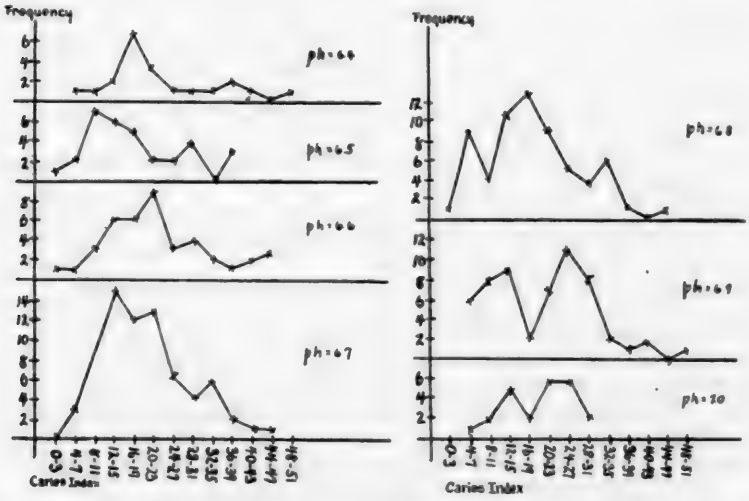

FIG. 4. Distribution of caries indices for various pH values

activity are desirable for future studies. This may shed new light on the effect of hyperacidity or hyper-alkalinity on the production or inhibition of dental caries.

SUMMARY

Colorimetric salivary pH determinations and the incidence of dental caries of 351 young male adults were studied. Extreme values of salivary pH reported by some investigators were not observed in our studies. No relationship between the incidence of dental caries and the pH of normal resting saliva was evident in the present study.

The author wishes to express his appreciation to Mr. M. Jacobs and Dr. H. Laden for their invaluable aid in the statistical study and to Dr. F. Cajori for his kindness in obtaining data for the pH readings and for many helpful suggestions.

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DO CALCIUM AND PHOSPHORUS IN SALIVA DIFFER SIGNIFICANTLY IN CARIES-FREE AND ACTIVE-CARIES GROUPS?[1]

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In a recent report, Becks, Wainwright and Young (1) stated that they were unable to find significant differences between caries-free and caries-active groups in the calcium and inorganic phosphate contents of unstimulated saliva. Karshan (2) had previously found significant differences in unstimulated saliva between 2 such groups in calcium and inorganic phosphate, and in certain other characteristics. The average calcium and phosphate values were higher for the caries-free than for the active-caries group. Becks, Wainwright and Young were of the opinion that the reported differences may have been due in part to a higher rate of flow in the active-caries group, and in part to chance variation in the selection of subjects.

It seems more likely that the failure of Becks, Wainwright and Young to confirm the author's findings may be due to fundamental differences in experimental conditions, such as the clinical criteria of caries activity in the subjects studied, the time of collection of saliva and the treatment of saliva preliminary to analysis. Becks and his collaborators themselves emphasized the "necessity of always examining the same type of saliva if comparable results are to be obtained" by different investigators.

In the author's investigations the manner of selecting cases, as previously described (2) was as follows: Each individual in the caries-free group had a complete set of natural teeth, free from cavities and fillings, as shown by thorough mouth examination and checked by radiograph. All doubtful cases, such as those having pits or fissures large enough to admit an explorer, were excluded from this group. In the arrested-caries group, no unfilled cavities were present; the fillings were small, few, occurred occlusally, and were prepared at least 5 years ago. The active-caries group included those who had active decay apparently of recent origin, associated with definite disintegration of enamel, accompanied by softened dentin. The number of cavities per person varied from a few to many; in subjects having only a few, proximal surfaces were involved. In these cases [all subjects in active-caries group], teeth other than those with large cavities had areas of decalcification, usually at the gingival margin. Individuals having large cavities, with firm, discolored dentin, were not included. The miscellaneous group included individuals who had cavities

[1] Received for publication October 27, 1941.

or fillings, but could not be placed in either the arrested- or active-caries groups. It should be emphasized that the presence of a large number of cavities did not necessarily place a subject in the active-caries group, and that all active-caries cases had areas of enamel decalcification, usually at the gingival margin, in addition to the cavities as described above.

In the investigation of Becks, Wainwright and Young caries activity was "indicated by the finding of new enamel caries as determined by periodic clinical examinations during the time in which the salivary analyses were made." Their subjects had a "large number of cavities" and high salivary *Bacillus acidophilus*

TABLE I

*Mean values for unstimulated saliva of caries-free and caries groups**

TYPE OF CASE	NO. OF CASES	NO. OF SETS OF ANALYSES	AGE	TOTAL CALCIUM	INORGANIC PHOSPHORUS	RATE OF FLOW
			years	*mg./100 cc.*	*mg./100 cc.*	*cc./hr.*
Caries-free	21	43	18.6	6.5	18.5	24.4
Arrested-caries	13	29	21.0	6.4	18.5	24.6
Active caries	24	47	18.5	5.7	14.6	27.1
Miscellaneous caries	20	35	22.1	6.3	18.7	22.1
Difference between mean values of caries-free and active-caries groups divided by the standard error of the difference, or $\frac{D}{\sigma D}$				2.9	3.5	1.1
Odds that the difference between the caries-free and active-caries groups is not due to chance				267 to 1	2149 to 1	2.7 to 1
Difference between mean values of caries-free and miscellaneous groups divided by the standard error of the difference, or $\frac{D}{\sigma D}$				0.6	0.2	0.7
Odds that the difference between the caries-free and miscellaneous groups is not due to chance				1 to 1	Less than 1 to 1	1 to 1

* The calcium and phosphorus values are from the author's previous publication, *J. D. Res.:* (**18**: 395, 1939).

counts. No indication is given of the interval represented by the "time in which salivary analyses were made." This description leaves the possibility entirely open that Becks' "caries" group comprised or consisted largely of cases which would have been designated "miscellaneous" by the present author. The data in Table I show that no significant differences were found by the author between the miscellaneous and the caries-free groups in the average calcium and phosphate contents of unstimulated saliva. This may be one reason for the negative findings of Becks, Wainwright and Young. Since the writer did not make bacteriological examinations, his miscellaneous group cannot be compared with Becks' caries group in this respect.

It may be pointed out also that in the author's investigation, analysis was made of whole saliva collected one to three hours after breakfast. Becks,

Wainwright and Young collected saliva before breakfast and centrifuged it before analysis was made. It is known that centrifuged saliva differs in composition, particularly in its calcium content, from whole saliva (3, 4, 5). Apart from the advantages or disadvantages of collecting saliva before or after breakfast, or of determining calcium and inorganic phosphate before or after centrifuging, it is clear in view of the differences in experimental conditions employed by Becks and by the author, that the respective data are not fully comparable, and hence that no conflict can be demonstrated in the respective findings. Moreover, Becks and his co-workers are hardly justified in stating that their observations confirm the previous findings of Wach (6). On the contrary, Wach, in his study of unstimulated saliva of a rather small series (10 "immune" and 14 active-caries cases) found a higher average calcium value in the "immune" (6.9 mg. per 100 cc.) than in the active-caries group (6.3 mg. per 100 cc.). Again, Becks' statement, based on a study of unstimulated saliva, that "any suspicion of a relationship between salivary calcium and phosphorus values and dental decay must be abandoned," completely overlooks the fact that differences of statistical significance have been found between caries-free and active-caries groups in studies of paraffin-stimulated saliva (2, 7).

As mentioned before, Becks, Wainwright and Young stated that the differences in calcium and phosphate found by the author between the caries-free and active-caries group may have been due to a higher rate of flow of saliva in the caries group. While Becks has shown that very large differences in the rate of secretion are associated with variations in the calcium and phosphate contents of saliva, there is no evidence to suggest that the very small average difference in rate of flow between the author's caries-free and active-caries groups could account for the differences in calcium and phosphate of the magnitude reported. As shown in Table I, the difference in the rate of flow between the author's caries-free and active-caries groups is not significant.[2]

Becks *et al.* state further that the "fact that the ranges [of the two groups] overlap considerably, and, furthermore the observation that salivary calcium and phosphorus concentration (in mg. per cent) is dependent, to a large extent, upon the rate of secretion and the age of the individual," led them to believe that the statistically significant differences which the present author obtained were the result of chance variation in the selection of subjects. It has already been shown that it is unlikely that the small difference between the two groups in the rate of secretion could account for the differences in calcium and phosphate. Moreover, in view of the similarity of the average ages of the 2 groups, it is likewise unlikely that the age factor played a part in determining the differences obtained by the author. As for the overlapping of ranges in the 2 groups, it is well known that statistical methods such as that used by the author are specifically designed to evaluate the significance of variability of individual observations in a group. The overlapping of ranges of the 2 groups does not invalidate

[2] It may be noted, contrary to the assumption made by Becks, Wainwright and Young, that in the author's investigations no subject was rejected because of difficulty in obtaining a sufficient sample of saliva for analysis.

the significance of the mean differences, although it may well indicate that the composition of saliva is only one of a number of factors associated with dental caries.

The statistical method employed by the author is intended to evaluate the probability that a difference in the means of 2 groups is or is not due to change. The value 2.9, the ratio of the difference in means for calcium (between the caries-free and active-caries groups) to its standard error, indicates that the odds are 267 to 1 that the difference is not due to chance (8). Likewise the $D/\sigma D$ value of 3.5 for phosphorus means that the odds are 2149 to 1 that the difference between the 2 groups was not due to chance. Hence the statement of Becks, Wainwright and Young that the differences reported by the author are due to chance selection of subjects, is unlikely to be true. The likelihood that the differences are not due to chance is strengthened by the almost precise correspondence of values in the author's caries-free and arrested-caries groups.

It may be noted that among the reasons given by Becks, Wainwright and Young for taking issue with the author's findings, is the size of the groups studied: 21 caries-free and 24 active-caries subjects. Nevertheless, Becks' group cites in support of their own findings the data of Wach (who studied only 10 "immune" and 14 caries cases), and of White and Bunting (who studied only 12 and 13 cases in each group). It has already been noted that the group averages for calcium reported by Wach, even so, are not in agreement with those of Becks.

SUMMARY

An analysis in terms of the criticism offered by Becks, Wainwright and Young of the author's previous findings, that average values for calcium and inorganic phosphate of unstimulated saliva differ significantly in caries-free and active-caries groups, makes it clear that the data of the 2 groups of investigators are not comparable because of differences in experimental conditions. Hence the findings of Becks, Wainwright and Young cannot be said to be in conflict with those of the author.

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HUMAN SALIVA

XII. THE EFFECT OF FLAVORED ACTIVATORS ON SALIVARY FLOW AND CALCIUM AND PHOSPHORUS COMPOSITION[1]

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The effect of flavored activators on salivary flow and calcium and phosphorus composition has found little, if any, consideration thus far. In view of recent findings that Heidenhain's law does not apply to paraffin activation (5), the response of human salivary glands to various types of activators was tested. For this purpose, the rate of flow and the calcium and phosphorus values of resting saliva[2] were compared with those of A.S. of 4 individuals (2 females and 2 males) in good physical condition. They were 36, 40, 25 and 28 years of age, and are designated in the following tables as A, B, C and D, respectively. The activators used were: paraffin (A.1[3]), pure chicle (A.2), and gums of various flavors (A.3, 4 and 5). R.S. samples were collected before breakfast under conditions described previously (3, 4 and 5); activation immediately followed and consisted of chewing one activator for 3 successive periods of 450 chews each (averaging 7 minutes per period). In several instances, it was not possible to obtain a sufficient amount of saliva with 450 chews for analysis during the third activation period, which explains the omission of the third result in some figures. The procedure was carried out on 5 days, only one activator being used on each day. The rate of flow was determined and analyses made for total calcium (10) and inorganic phosphorus (11). The averages[4] for R.S. of the 4 individuals on 5 days showed that there was only a slight variation from day to day (Table I, Column 1).

ACTIVATOR 1 (PARAFFIN)

Paraffin has been the most popular salivary activator and was therefore studied first. Each of the subjects chewed one gram paraffin of uniform consistency. The average results for A.1 (as well as for A.2, 3, 4, 5) are given in Table I.

[1] Conducted under grants made by the Carnegie Corporation, the California State Dental Association, California Academy of Periodontology, Research Board of the University of California and the Good Teeth Council for Children, Inc. (Received for publication, July 7, 1941.)

[2] The initials R.S. and A.S. will be used to designate resting and activated saliva.

[3] The activators will be designated by the initial A, i.e., Activator 1 = A.1.

[4] Individual values are omitted in the tables; however, they are presented in *figs. 1* and *2*.

Rate of Flow: The average rate of flow of R.S. was 11.4 cc./hr. (Column 1). During the first period of activation, a striking increase of 961.5 per cent was noted, followed by a tendency to drop in the second and third periods from 961.5 to 895.8 and 767.3 per cent. The individual rates of flow of R.S. and paraffin A.S. in cc./hr. are shown graphically in *fig. 1-A*.

Calcium and Phosphorus: During the first period of activation, the calcium mg. per cent values decreased an *average* of 32.4 per cent below the R.S. level (Table I) and continued to drop during the second and third periods, averaging

TABLE I

Effect of activation on salivary flow and calcium and phosphorus content (averages of four individuals)

		CUBIC CENTIMETERS PER HOUR AND MILLIGRAMS PER CENT				MILLIGRAMS PER HOUR			
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
	ACTIVATOR	Resting saliva	Activated saliva Percentage change from resting saliva value			Resting saliva	Activated saliva Percentage change from resting saliva value		
			1 450 chews	2 450 chews	3 450 chews		1 450 chews	2 450 chews	3 450 chews
		cc./hr.	%	%	%	*mg./hr.*	%	%	%
Rate of Flow	1	11.4	+961.5	+895.8	+767.3				
	2	14.2	+412.0	+275.0	+232.4				
	3	15.2	+924.2	+440.6	+332.0				
	4	16.0	+974.0	+360.6	+352.2				
	5	13.2	+1,324.9	+586.5	+381.7				
		mg. %							
Calcium	1	5.99	−32.4	−40.1	−45.9	0.71	+623.6	+506.0	+385.3
	2	5.65	−24.6	−12.2	−21.4	0.82	+292.7	+236.6	+161.2
	3	5.44	+14.7	−6.4	−17.3	0.85	+1,056.9	+403.8	+257.6
	4	5.18	+8.4	−1.4	−17.1	0.86	+1,062.7	+344.2	+271.4
	5	5.74	+1.3	−14.5	−22.6	0.79	+1,271.7	+450.9	+260.2
Phosphorus	1	21.0	−39.7	−48.3	−46.3	2.28	+518.0	+354.8	+322.0
	2	18.3	−26.0	−26.1	−21.0	2.48	+283.1	+173.7	+160.0
	3	18.6	−36.6	−38.0	−34.8	2.71	+536.2	+237.0	+181.8
	4	17.7	−26.3	−33.6	−30.3	2.79	+690.6	+203.8	+212.8
	5	20.8	−41.1	−43.1	−38.6	2.60	+699.6	+277.9	+192.2

40.1 and 45.9 per cent. The inorganic phosphorus showed similar decreases, averaging 39.7, 48.3 and 46.3 per cent. The individual mg. per cent changes from R.S. to A.S. are shown in *fig. 1-B*. These findings, showing decreased mg. per cent values with increased secretion, confirm the earlier observation that Heidenhain's law does not apply to the mg. per cent calcium and phosphorus content of human paraffin A.S. (5). In contrast to this, the mg./hr. values (Table I Columns 5, 6, 7 and 8) increase decidedly during the first period of activation, followed by a definite and continued decline in the second and third samples, however, never falling as low as the original R.S. level.

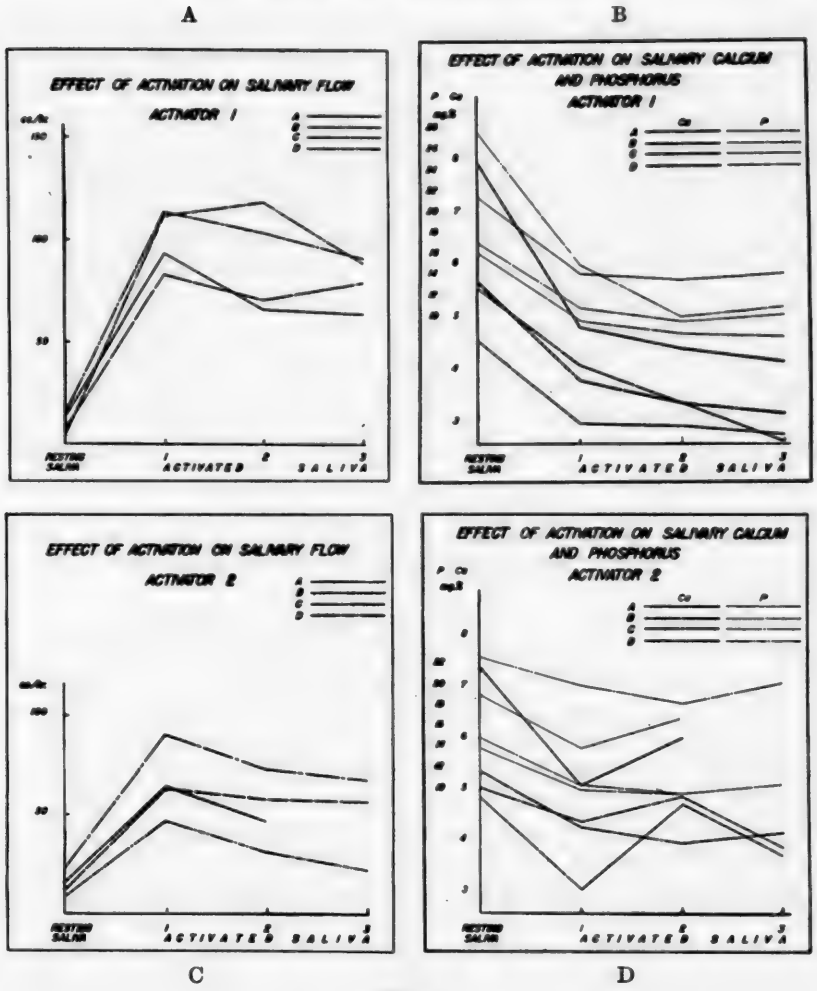


FIG. 1

ACTIVATOR 2 (PURE CHICLE)

Pure chicle[5], the second activator, was used because it is the tasteless, unflavored base of A.3, 4 and 5, and is slightly harder and tougher than paraffin. The amount of chicle and method of activation were identical with those used for paraffin.

Rate of Flow: During the first activation period the rate of flow increased; however, the average was less than half that obtained with A.1 (412.0 per cent as against 961.5 per cent, Table I). Again, in the second and third activated samples, a decrease of rate of flow was found, which was, however, far less pro-

[5] Pure chicle was placed at our disposal through the courtesy of the Wm. Wrigley Jr. Co.

nounced than with A.1. A comparison of *figs. 1A* and *C* shows a decided difference between the highest peaks reached at each of the 3 activation periods for these 2 activators.

Calcium and Phosphorus: During the first activation period, a decrease in mg. per cent values for calcium and phosphorus content again occurred (Table I, Column 2); however, this was far less pronounced than the decrease observed with A.1. Contrary to the decrease of rate of flow during the second and third periods of activation, calcium tended to increase (Table I, Columns 3 and 4, and *fig. 1D*). The striking difference between the individual percentage changes of calcium and phosphorus mg. per cent values obtained by A.1 and those obtained by A.2 is shown graphically in *fig. 3A*. The *mg./hr.* values for calcium and phosphorus found during activation with A.2 (Table I) were less than half of those for A.1. Since both paraffin and pure chicle are unflavored, it must be surmised that the difference in their consistencies is the primary factor which could account for the striking variation in the response of the salivary glands.

ACTIVATORS 3, 4 AND 5

The response to A.3, 4 and 5 (gums of various flavors) were similar although not identical. The findings (Table I) for all 3 activators, will be considered together.

Rate of Flow: The initial percentage increase of rate of flow due to activation with A.3 and 4 was approximately the same as that obtained with paraffin. During the second and third activation periods, however, they dropped to half the values for A.1 (Table I, Columns 3 and 4). Even though the initial percentage increase of rate of flow resulting from chewing A.3 and 4 reached approximately the same level as for A.1, the cc./hr. values (*figs. 2A*, *2C*, *1A* and *4C*) of the former are bound to be much higher. As observed for A.1 and 2 (*figs. 1A* and *1C*), the greatest initial increase of rate of flow as a result of activation was found during the first period; however, the results obtained throughout the three activation periods for A.4 were more nearly uniform for all four subjects.

A.5 occupies a unique position because the initial increase from R.S. to A.S., averaging 1,324.9 per cent (Table I, Column 2, *fig. 2C*), was decidedly higher than any obtained from A.1, 2, 3 or 4. This increase was followed by a considerable drop in the second and third samples to 586.5 and 381.7 per cent.

Calcium and Phosphorus: In contrast to the decrease in mg. per cent values for calcium and phosphorus obtained with A.1 and 2 (Table I, *figs. 1B*, *1D* and *4D*) during the first activation period, a remarkable increase of calcium occurred in all instances with A.3 and 4 (*figs. 2D* and *2E*). When A.5 was used (*fig. 2F*), 2 individuals responded with an increase, while the other 2 showed a decrease similar to the response with A.1 and 2. The general increase of mg. per cent calcium during the first activation period was followed by a tendency to decrease during the second and third periods. Attention may be called to the fact that individuals B and D gave almost the same values in each period of activation; A and C also showed similar close values, however, at lower levels than those of B and D.

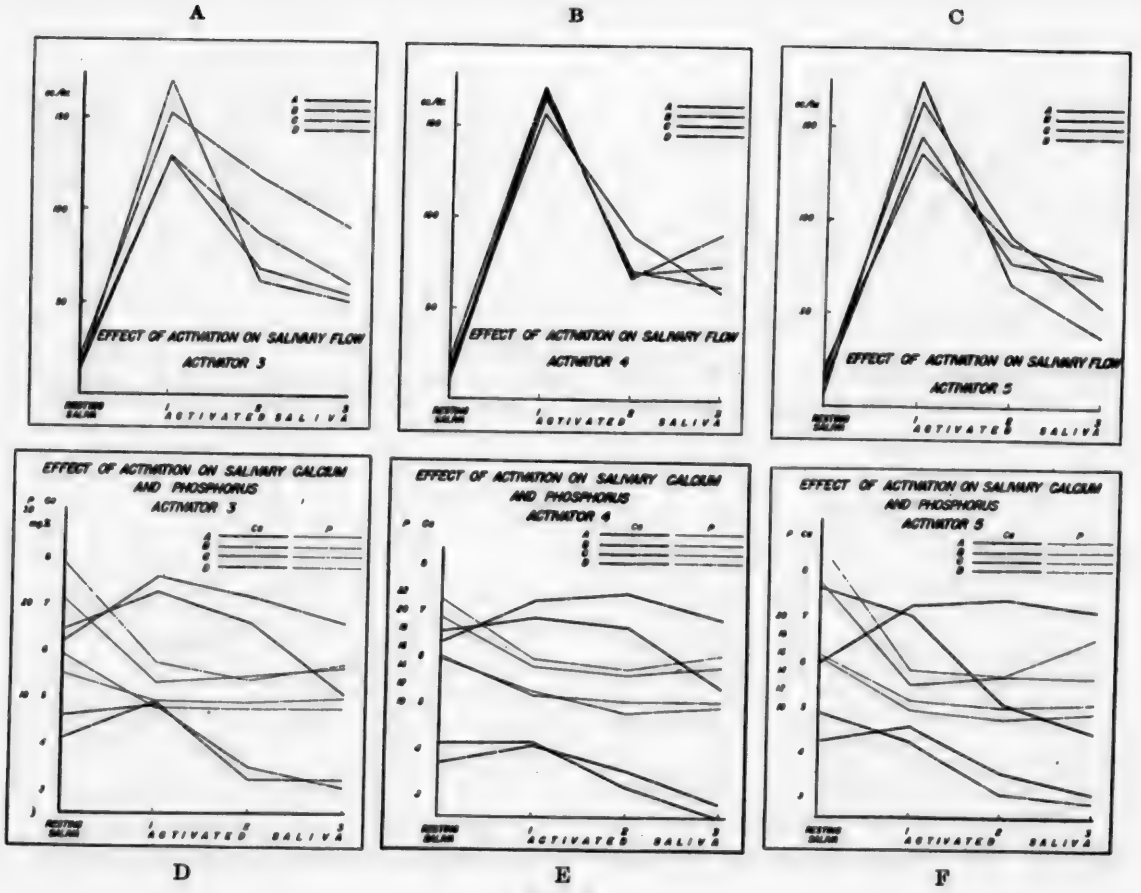
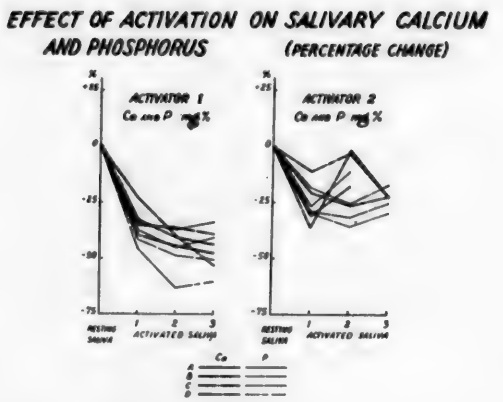
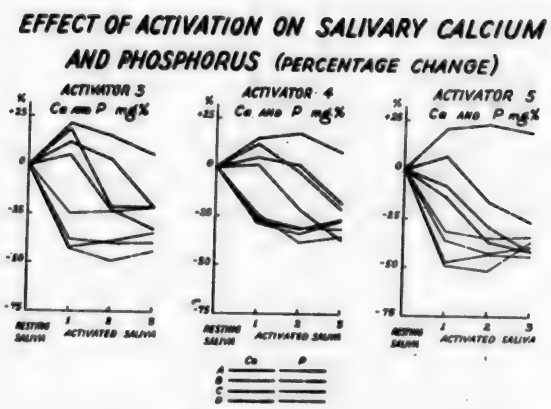

A
B
C
EFFECT OF ACTIVATION ON SALIVARY FLOW
ACTIVATOR 3
EFFECT OF ACTIVATION ON SALIVARY FLOW
ACTIVATOR 4
EFFECT OF ACTIVATION ON SALIVARY FLOW
ACTIVATOR 5
EFFECT OF ACTIVATION ON SALIVARY CALCIUM AND PHOSPHORUS
ACTIVATOR 3
EFFECT OF ACTIVATION ON SALIVARY CALCIUM AND PHOSPHORUS
ACTIVATOR 4
EFFECT OF ACTIVATION ON SALIVARY CALCIUM AND PHOSPHORUS
ACTIVATOR 5
D
E
F

FIG. 2

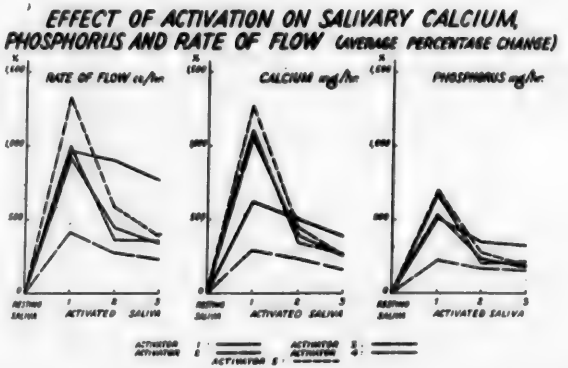
A


EFFECT OF ACTIVATION ON SALIVARY CALCIUM AND PHOSPHORUS (PERCENTAGE CHANGE)
ACTIVATOR 1
ACTIVATOR 2
Ca AND P mg%
RESTING SALIVA
ACTIVATED SALIVA
Ca
P

B


EFFECT OF ACTIVATION ON SALIVARY CALCIUM AND PHOSPHORUS (PERCENTAGE CHANGE)
ACTIVATOR 3
ACTIVATOR 4
ACTIVATOR 5
Ca AND P mg%
RESTING SALIVA
ACTIVATED SALIVA
Ca
P

C


EFFECT OF ACTIVATION ON SALIVARY CALCIUM, PHOSPHORUS AND RATE OF FLOW (AVERAGE PERCENTAGE CHANGE)
RATE OF FLOW cc/hr
CALCIUM mg/hr
PHOSPHORUS mg/hr
RESTING SALIVA
ACTIVATED SALIVA

D

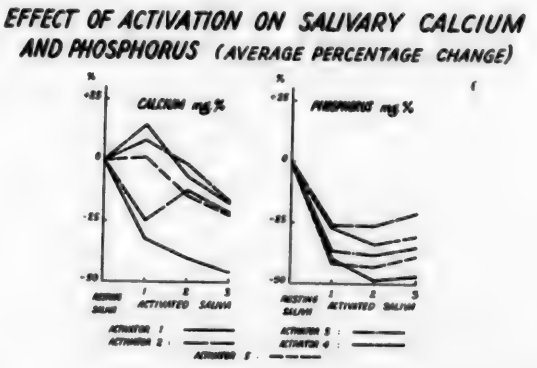

EFFECT OF ACTIVATION ON SALIVARY CALCIUM AND PHOSPHORUS (AVERAGE PERCENTAGE CHANGE)
CALCIUM mg%
RESTING SALIVA
ACTIVATED SALIVA

FIG. 3

This peculiar response of calcium secretion to A.3, 4 and 5, which showed for the first time an increase in mg. per cent values with increased rate of flow (following Heidenhain's law), was not found to be true for phosphorus secretion. Whereas the calcium mg. per cent values increased during the first period, the phosphorus values decreased as with A.1 and 2. In *figs. 2D*, *E* and *F*, it can be noted that there were much greater differences between the final calcium values of the 4 individuals than between the corresponding figures for phosphorus.

The percentage change of calcium and phosphorus values produced by A.3, 4 and 5 is shown graphically in *figs. 3B* and *D*. The mg./hr. values revealed an increased secretion of calcium and phosphorus paralleling the increased rate of flow as the result of activation with A.3, 4 and 5. However, they were very much higher than those obtained with A.1 and 2, at least, for the first activation period (Table I, Column 5, and *fig. 3C*). A sharp drop can be noted in the second and third periods.

TABLE II

Calcium content of activators before and after chewing

		ACTIVATOR				
		1	2	3	4	5
		mg. Ca. per gram	mg. Ca. per gram	mg. Ca. per piece	mg. Ca. per piece	mg. Ca. per piece
Before chewing		0.00 0.00	13.00 13.00	8.90 8.75 8.75	5.55 5.55	5.10 5.10 5.00
After Chewing	150 Chews			8.65		5.10
	300 Chews			8.30		5.05
	450 Chews			8.40		4.70
	1350 Chews	0.21 0.00 0.00	12.60		4.80	4.80

Analysis of Activators. The increase of mg. per cent calcium as the result of activation with A.3, 4 and 5 raised the question as to whether the calcium was introduced by the flavored activators or whether it came from the salivary glands, possibly influenced by the various flavors. For this reason, all activators were dry-ashed and analyzed for calcium both before and after chewing (Table II).

Analysis of the 5 activators revealed a varying content of calcium. As would be expected, A.1 (paraffin) did not contain any, whereas, A.2 (pure chicle) contained as much as 13.00 mg. calcium per gram; A.3, an average of 8.75; A.4 = 5.55; and A.5 = 5.10 milligrams Ca. Since the formulae of these gums are not known, it is possible that quantitative and qualitative variations in the chicle content of A.3, 4 and 5 may account for differences shown by these analyses. Following various periods of chewing, the ash analyses of the chewed activators revealed a slight loss of calcium in A.2, 3, 4 and 5 (Table II). This

loss did not, however, express itself in an increase of the salivary calcium content (Table I) when A.2 was used.

For this reason, A.3 and 5 were especially tested for calcium loss as compared with the calcium increase of saliva samples during successive activation periods. Three analyses each were made of A.3 and 5 in order to establish their calcium content before use. Following the collection of a sample of R.S. from one individual, 3 pieces of A.3 of equal weight and consistency were chewed: the first 150, the second 300 and the third piece 450 times. The saliva for each period and piece was collected separately. The 3 chewed pieces were analyzed, as well

TABLE III

Analyses of saliva and activators before and after chewing

	ACTIVATOR 3					ACTIVATOR 5				
	(1)		(2)	(3)		(1)		(2)	(3)	
	Activator	Resting saliva				Activator	Resting saliva			
	Calcium	Time	Rate of flow	Ca	P	Calcium	Time	Rate of flow	Ca	P
	mg.	*min.*	*cc./hr.*	*mg. %*	*mg. %*	*mg.*		*cc./hr.*	*mg. %*	*mg. %*
Unchewed Activator	8.75	17	31.1	6.45	16.4	5.00	18	30.0	5.85	16.8
		Activated saliva					Activated saliva			
Chewed Activator (150 Chews)	8.65	2¼	288.0	8.85	12.5	5.10	2¼	252.0	7.15	10.8
Chewed Activator (300 Chews)	8.30	4½	245.0	8.55	10.5	5.05	4½	241.0	6.90	9.7
Chewed Activator (450 Chews)	8.40	6	243.0	7.70	10.8	4.70	7	214.0	6.45	10.3

TABLE IV

Effect of chewing on the calcium content of activators and saliva

	ACTIVATOR 3		ACTIVATOR 5	
	Calcium loss of activator	Calcium increase of saliva	Calcium loss of activator	Calcium increase of saliva
	mg.	*mg.*	*mg.*	*mg.*
150 Chews	0	0.42	0	0.30
300 Chews	0.45	0.60	0	0.47
450 Chews	0.35	0.58	0.30	0.53

as the sample of R.S. and the 3 samples of A.S. This procedure was duplicated on another day for A.5. The individual results are given in Table III, and the loss and recovery of calcium calculated from them are presented in Table IV.

It can be noted that the salivary calcium content increased in every instance as a result of activation by these flavored gums in spite of the fact that in 3 of the 6 analyses, the chewed activator failed to show a loss of calcium. In those instances where a loss of calcium from the activator was encountered, the increase in salivary calcium surpassed by far the amount lost by the activator. The question now arises as to whether the calcium increase is derived from

deposits on the surface of the teeth or from particles in the interdental spaces which became dislodged during chewing of the activators. The single fact that chewing of paraffin and chicle did not increase the calcium content may to some extent indicate that this possibility can be ruled out. However, it must be kept in mind that the consistency of paraffin and pure chicle vary slightly from that of A.3, 4 and 5.

Since the temporary increase of salivary calcium occurred solely during activation with flavored gums, it can be concluded that this increase is the result of an elective response of the salivary glands to these flavors. The apparent paradox of increase in calcium accompanied by a decrease in phosphorus may possibly be attributed either to a response of different cells within the same or within different salivary glands, to activation by different nerves, or to changes within the granules of the individual salivary cells (5). Up to the present time, these are the only conditions under which Heidenhain's law seems to apply to the calcium content of human salivary secretions.

COMMENT AND CONCLUSIONS

In order to obtain more information about the intricate changes that occur during the process of salivary secretion in human beings under resting and activated conditions, one must bear in mind the fundamental observations of Ludwig, Heidenhain, Pavlov, Babkin and their schools. In a survey of the dental literature, one is struck by the lack of attention to these fundamentals of salivary secretion and by the meager knowledge that is available today of the effects of activation on salivary composition in human beings. Our interest in this matter arose from a desire to learn the various influences that may lead to changes in the composition of R.S. and A.S. under normal and pathologic conditions, since little information is available today on the factors that determine the fluctuations of salivary composition.

In previous experiments, as well as in those presented here, it has been found that the fundamental law of Heidenhain, which was established mainly by observations on animals, does not apply to all of the solid elements of mixed human saliva. It was noted that individuals with a fast flowing saliva had much lower calcium and phosphorus concentrations than did those with a slowly flowing saliva; and, in the same individual, it was found that R.S. was more concentrated than A.S., in spite of the faster rate of flow of the latter. Because of these findings, experiments were performed on human subjects to test the response to various types of salivary activators. The rate of flow and the total calcium and inorganic phosphorus content of R.S. and A.S. of the same individuals were compared. The glands were activated by paraffin, pure chicle, and flavored gums.

The analytical results of paraffin activated saliva served as a basis for a comparison with those obtained by other activators. The rate of flow was increased considerably by paraffin activation which was followed during succeeding stimulations by a slight decline. With the increase of salivary flow, the mg. per cent calcium and phosphorus values decreased initially followed by a further drop.

These observations are in contradiction to Heidenhain's law. Quite in contrast to the mg. per cent calculations, the mg./hr. values for calcium and phosphorus increased greatly and showed a subsequent tendency to decline.

Chicle, the unflavored tasteless base of activators 3, 4 and 5, caused a far smaller increase in rate of flow than did paraffin. The calcium and phosphorus mg. per cent values decreased initially as with paraffin, but to a much lesser degree and with a tendency of the third sample to return to the R.S. level. The mg./hr. calcium and phosphorus values followed a trend similar to that of paraffin A.S.; however, they did not show as great an increase. The explanation for the difference between the paraffin A.S. and chicle A.S. may be that chicle is tougher and harder in consistency.

At first, all the flavored gums induced a high rate of flow which was far above that for paraffin; subsequently, the rates fell considerably, even to a level between those for paraffin and chicle.

An extraordinary response in respect to calcium secretion was noted with activators 3, 4 and 5. In general, these activators caused an increase of mg. per cent calcium content, quite in contrast to the observations with A.1 and 2. For the first time, the results followed Heidenhain's law for electric stimulation. In subsequent periods of activation, the calcium values fell below the R.S. level, but not half as far as when paraffin was used. The phosphorus content decreased with these activators as during paraffin activation.

The mg./hr. calcium values increased at first to double the level obtained by paraffin activation, but fell considerably in the second and third periods. Phosphorus in mg./hr. increased much less than calcium, which biologically may have some significance.

Analyses of the activators revealed no calcium in the paraffin either before or after chewing. Chicle and all of the gums, on the other hand, contained comparatively large amounts of calcium and after chewing showed a slight loss, thus indicating a possible source of the increase of salivary calcium. However, analyses of activators before and after chewing and of the A.S. produced did not show a sufficient loss of calcium from the activators to account for the increase in salivary content.

It may be concluded, therefore, that the increase of calcium in activated saliva is the result of an elective response of the salivary glands to the different flavors of the gums. Furthermore, these observations indicate that the calcium and phosphorus concentrations of mixed human saliva can be changed as desired, by various types of activation. It is conceivable that, with a greater or lesser amount of calcium and phosphorus available in the blood stream and lymphatic tissue under normal and pathologic conditions, the concentration of saliva may change frequently due to this elective response of the salivary glands.

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INCREASE IN HARDNESS OF DENTIN ON DRYING[1]

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The 18 teeth used, mostly incisors and canines, with a few molars, were taken, freshly extracted, at random from the clinic in the Strong Memorial Hospital, and kept in 5 per cent formaldehyde in tap water until cut. Each tooth was held in a plaster mounting while a sagittal section about 1 mm. in thickness was cut by a circular steel saw. When not being tested, each tooth section was kept in tap water.

To make the microcuts, each slab section was mounted in plasticine on a microscope slide; precautions were taken that the surface of the slab was parallel to the surface of the slide. The slide was then placed on the microcharacter stage,[2] and a microcut made in the coronal part of the dentin. Using a filar micrometer at a magnification of about 1000 diameters, the width of the microcut was measured at about 20 places along the cut; the microhardness was determined from these data.

After determining the microhardnesses of the moist slabs, they were left in uncapped bottles to be desiccated by the air at room temperature for a period of 6–12 weeks. The dried teeth were rubbed with a very fine emery paper and subsequently polished with a moistened abrasive powder on a buffer wheel. The dried, polished sections were again mounted and cut as before; the widths of the new microcuts were measured, and the microhardnesses were calculated.

Effect of Polishing: In order to determine the effect of polishing on the hardness, an additional 10 teeth were taken at random, keeping each tooth moist and unpolished. The average microhardness was determined, based upon 10 readings. Subsequently, the teeth were polished while each tooth was still moist and then the microhardness was redetermined from a second microcut made in about the same part of the tooth. The teeth were then dried in an oven at 105°C. for 44 hours. Their microhardnesses were again determined (Table I) from a third microcut.

The data in Table I, based upon 10 readings for each microcut, include the average widths (in μ) of each cut on (a) moist and unpolished, (b) moist and

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[2] Hodge, H. C. *J. D. Res.* **15**: 278, 1936.

polished, and (c) dried and polished sections. In addition, the average calculated hardness for each cut has been recorded. It may be seen from this

TABLE I

Width and microhardness data on polished and unpolished dentin

TOOTH NO.	WIDTH (MICRONS)				MICROHARDNESS				
	Moist		Dry	Difference	Moist		Dry	K	%
	Unpolished	Polished			Unpolished	Polished			
1	9	9	7	2	123	123	204	81	39
2	9	9	6	3	123	123	278	155	55
3	9	9	7	2	123	123	204	81	39
4	8	8	6	2	156	156	278	122	43
5	8	8	6	2	156	156	278	122	43
6	9	8	7	1	123	156	204	48	24
7	8	8	8	0	156	156	156	0	0
8	8	9	7	2	156	123	204	81	39
9	8	8	7	1	156	156	204	48	24
10	8	9	8	1	156	123	156	33	21
Average					143	140	217	77	33

TABLE II

Width and microhardness data on moist and dry dentin

TOOTH NO.	WIDTH (MICRONS)						MICROHARDNESS			
	Moist		Dry		Difference		Moist	Dry	Difference	
	No. readings	Average	No. readings	Average		%	Average K	Average K	K	%
1	19	9	26	8	1	12	123	156	33	21
2	13	10	20	9	1	11	100	123	23	19
3	10	14	20	9	5	56	51	123	72	59
4	15	12	20	9	3	33	70	123	53	43
5	20	12	20	9	3	33	70	123	53	43
6	20	13	20	11	2	18	59	83	24	29
7	20	14	20	9	5	56	51	123	72	59
8	20	13	20	8	5	62	59	156	97	62
9	20	13	20	11	2	18	59	83	24	29
10	20	12	18	11	1	9	70	83	13	16
11	20	11	20	9	2	22	83	123	40	33
12	20	12	20	9	3	33	70	123	53	43
13	20	11	20	8	3	38	83	156	73	47
14	20	13	20	8	5	62	59	156	97	62
15	20	12	20	8	4	50	70	156	86	55
16	22	11	20	8	3	38	83	156	73	47
17	17	10	20	8	2	25	100	156	56	36
18	20	11	20	9	1	11	83	123	40	33
Average		12		9	3	33	75	129	54	42

table that the effect of polishing on the hardness was negligible and that the effect of drying was to increase the hardness.

Effect of Drying: The data in Table II include the number of width readings for each microcut, the average widths (in μ) of each cut on moist and dried sections, and the average calculated hardness for each cut.

SUMMARY

(1) In each tooth, the average width of the microcut was less for the dried dentin than for the moist.

(2) The changes in the dentins on drying were as follows: 4 teeth had a decrease in microcut widths of 5μ; 1 tooth had a decrease of 4μ; 5 teeth had a decrease of 3μ; 4 teeth had a decrease of 2μ; and 4 teeth had a decrease of only 1μ.

(3) The average width of the microcuts for all moist dentins was 12μ, for all the dried dentins was 9μ (average difference—3μ; average per cent difference—33).

(4) The dentin microhardnesses according to the Bierbaum scale was increased on drying from 75 to 129 (average increase—54; average per cent increase—42).

ABRASION OF DENTAL GOLD ALLOYS[1]

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It is the opinion of certain dentists (1) that hard gold alloys are not indicated for inlays where the softer alloys will do as well because the abrasion resistance of the former is likely to be greater than that of the tooth enamel, thus creating "high spots" which may cause an occlusal trauma. On the same basis, the idea is prevalent that an inlay should not be given a hardening heat treatment (2) because of the increased hardness which results. The present research presents data on the effect of Brinell hardness and heat treatment on the abrasion resistance of certain dental gold alloys.

Six gold alloys were chosen for study. As may be noted from Table I, alloys B and F are *Type A* alloys (1); A, C and D are *Type B* or *Type C* alloys. The specific gravity of the various golds is also given in the table, together with that of tooth enamel. The Brinell hardness number was determined with the usual micro-Brinell equipment, and the specific gravity was obtained by the water displacement method with the use of a Jolly balance.

Cast specimens of each alloy, approximately 1 x 20 x 25 mm. were used for the abrasion test. Sound enamel from approximately 25 extracted teeth was also tested. The dentin was carefully cut from the enamel, and the pieces nearest approaching a plane surface were mounted in bakelite; they were then ground flat so that an even abrasion could be obtained. The area of abrasion was approximately 3.3 cm.^{2} for the gold alloys, and 1.7 cm.^{2} for each of the two enamel specimens employed.

A Pepsodent abrasion testing machine (3) was adapted for the test, by the removal of the toothbrushes and the substitution of soft rubber erasers for applying the abrasive. The rate of abrasion was 148 strokes per minute, a stroke being one back and forth movement. All specimens were subjected to 100,000 strokes for each determination. The abrasive agent employed was 50 grams of 200-mesh carborundum powder suspended in 30 cc. of U.S.P. glycerine. The mixture was renewed after every 5,000 strokes. The results are presented in Table II.

The heat treatments indicated are those specified by A.D.A. Specification No. 7 for Wrought Gold Wire Alloys (4). According to Table II, the hardening heat treatment increases the amount of abrasion for every gold alloy except F, which is not amenable to heat treatment (Table I). On the basis of the areas

[1] Read at the 18th General Meeting of the International Association for Dental Research, Philadelphia, March 13 and 14, 1940. (*J. D. Res.* **19**: 299, 1940). (Received for publication April 15, 1940; revised by author September 13, 1941).

of the abraded surfaces previously given, the enamel is more than twice as resistant to abrasion as the most abrasive resistant gold alloy C (softened).

The increase in abrasiveness upon hardening by heat treatment, over the softened condition of gold alloys, is possibly due to the greater ductility of the latter. Presumably the greater ductility allows a surface flow or burnishing which decreases the abrasive action. However, ductility is probably not the only factor involved.

Table III shows the constants for the ratio of the abrasion losses given in Table II to the respective Brinell hardness values in Table I. Neglecting the

TABLE I

Brinell Hardness Number and Specific Gravity

ALLOY	BRINELL HARDNESS NUMBER		SPECIFIC GRAVITY
	Softened	Hardened	
A	82	106	15.3
B	68	116	16.3
C	78	91	15.0
D	84	152	12.3
E	91	121	14.0
F	40	43	17.4
Enamel	—	—	2.96*

* Manly, R. S., Hodge, H. C., and Ange, L. E., J. Dent. Res., **18**: 204, 1939.

TABLE II

Loss in Weight (mg.) per 10,000 Abrasive Strokes

ALLOY	SOFTENED	HARDENED	INCREASE
	mg.	mg.	%
A	398.1	543.1	37
B	339.3	442.4	30
C	297.1	517.9	74
D	245.8	357.2	45
E	296.6	345.7	17
F	339.7	348.8	3
Enamel		61.6	

constants for alloy F, it is evident that the remaining alloys are of two classes in the softened condition as regards proportionality between abrasion loss and Brinell hardness number; alloys A and B have similar constants of proportionality, as do alloys C, D and E, but the constants are different for the two classes. This condition does not prevail after the hardening heat treatment. The significance of these relationships is not clear on the basis of the available data.

If the constants are compared for the individual allows in the softened and hardened condition, it is possible that a proportionality may exist with alloys A, D and E at least. It has been shown (4) that the Vickers Hardness Number may be in general inversely proportional to the elongation of a given gold wire alloy for different heat treatments. The proportionality in Table III therefore

suggests that the ductility of an alloy may be inversely proportional to its susceptibility to abrasion.

When the constant of proportionality is determined for the abrasion loss to specific gravity, a definite proportionality is found between the two properties for the alloys in the "softened" condition and possibly for the enamel[2], as shown in Table IV, but no such proportionality is evident in the "hardened" condition of the alloys.

The nature of the transformation involved will obviously vary according to the composition of the alloy; furthermore, the rate of atomic diffusion will also vary for the same reason. Inasmuch as all of the alloys were hardened by heat

TABLE III

Ratio of Abrasion Loss to Brinell Hardness Number

GOLD ALLOY	SOFTENED	HARDENED
A	4.9	5.1
B	5.0	3.8
C	3.8	5.7
D	2.9	2.3
E	3.2	2.9
F	8.5	8.1

TABLE IV

Ratio of Abrasion Loss to Specific Gravity

ALLOY	SOFTENED	HARDENED
A	26	35
B	21	27
C	20	34
D	20	29
E	21	25
F	20	20
Enamel	21	

treatment in the same manner, no proportionality should necessarily be expected in this condition between either specific gravity or Brinell hardness number and amount of abrasion.

SUMMARY AND CONCLUSIONS

The abrasion of six dental gold casting alloys, in both the "softened" and "hardened" condition, and tooth enamel has been determined. The conclusions may be summarized as follows:

1. Hardening of the gold alloys by heat treatment decreased their resistance to abrasion.

2. The tooth enamel was more than twice as resistant to abrasion as the most abrasive resistant alloy.

[2] Possibly this relationship is a coincidence since the surface area abraded is not the same as for the gold alloys. Further study is needed in this regard.

3. A proportionality between the amount of abrasion and the Brinell hardness number was indicated for five of the alloys in the softened condition.

4. In the case of 3 alloys, the amount of abrasion was directly proportional to the Brinell hardness number when the alloys were compared individually in the "softened" and "hardened" conditions, a fact which suggests a relation between ductility and amount of abrasion.

5. The amount of abrasion of the gold alloys after an anneal was directly proportional to their respective specific gravity values, but this relation did not generally obtain after age hardening, a condition which may possibly be explained upon the basis of varying composition and degrees of atomic diffusion of the alloys under a standardized heat treatment.

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A ROENTGEN-RAY ABSORPTION STUDY OF HEREDITARY OPALESCENT DENTIN[1]

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Several years ago a quantitative method was devised whereby the roentgen ray absorption of dental calcified tissues could be measured and results reproduced within an error range of 5 per cent (1). Since that time it has been employed to show changes in the inorganic content of dentin under varying physical (2, 3) and pathological conditions (4, 5). Van Huysen, from a study of 160 specimens, concluded that the average roentgen ray absorption of 1 mm. thickness of normal dentin is equivalent to that of 0.94 mm. of pure aluminum (6). The roentgen ray absorption properties of hereditary opalescent dentin are reported in this paper. X-ray absorption values have been determined experimentally and compared with a calculated, theoretical value and the value for normal dentin given by Van Huysen.

EXPERIMENTAL

The dentin of 15 teeth extracted from the mouth of Elmer S. Jr. provided the experimental material for this study. Typical findings for opalescent teeth (odontogenesis imperfecta), e.g. decreased root size, obliterated pulp canals, and selective loss of coronal structure by attrition and caries were disclosed by dental radiographs (7). Complete details of other aspects of this case have been reported extensively (7, 8).

A longitudinal slab about 1 mm. thick was removed from each tooth. These sections were ground planoparallel by the Precision Optics Department of the Bausch and Lomb Optical Company. The final thicknesses of the individual slabs were from 0.3 mm. to 0.6 mm. but the variation in thickness in any one slab did not exceed ± 0.01 mm. Throughout the experiment every effort was made to prevent drying of the slabs which, when not used, were kept in individual, small, screw capped bottles.

The prepared slabs were divided into 3 groups, each of which was placed on a film along with aluminum and alloy step tablets (penetrometers) and exposed in triplicate at 30 kv., 100 ma., for 2 seconds with a target-film distance of 53 inches. Such an exposure is reproduced in *fig. 1*. Any tube which produced x-rays of uniform intensity over the field of exposure at the desired kilovoltage could have been employed. In this laboratory, a Machlett CYS double line

[1] Presented at the 19th General Meeting of the International Association for Dental Research, St. Louis, March 15–16, 1941 (*J. D. Res.* **20**: 257, 1941). Received for publication October 21, 1941.

focus tube with the aluminum filter removed has proven satisfactory, and is used for all such exposures. A study of density values in relation to the exposure time at 30 kv. in connection with Eastman duplitized no-screen x-ray film, indicated that it was useful to record the various intensities of roentgen radiation transmitted by the slabs, and that the range of densities obtained through dentin at the given exposure lay along the straight-line portion of the "characteristic" curve (10). This condition was imperative if film density was to indicate the real intensity of tissue-transmitted radiation.

Before making exposures, the films were wrapped in light-impermeable black paper such as that in which they are generally enveloped when received from the

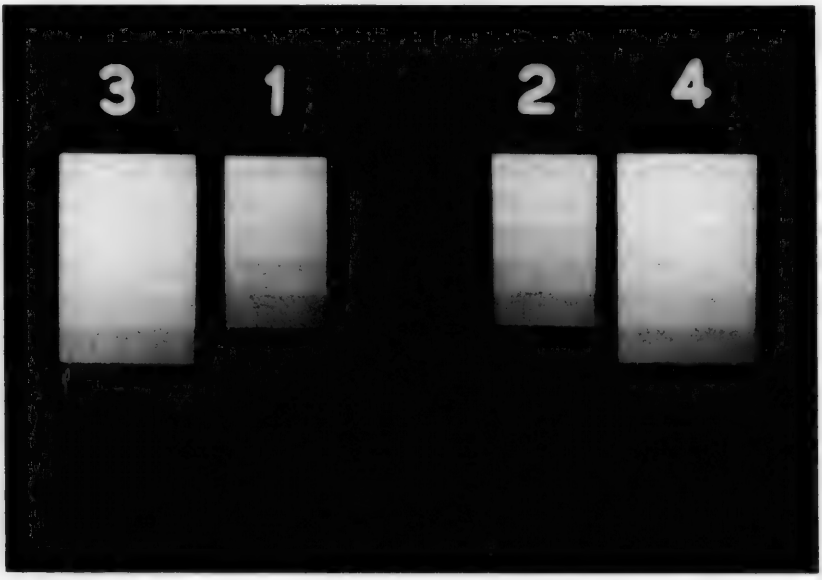

FIG. 1. X-ray exposure of group of opalescent tooth slabs to determine their absorption values. 1 and 2 are aluminum, and 3 and 4, alloy penetrometers. Slab at bottom was cut from normal tooth and is about same thickness as others.

manufacturer. Ordinary cardboard film holders or cassettes were not satisfactory because they do not absorb the roentgen ray uniformly. Films 8 x 10 inches in size were convenient since the triplicate exposure of groups of 5 or 6 tooth slabs may easily be made on one of them.

Development required 5 minutes (accurately timed) in a freshly prepared x-ray developer with constant agitation at 65° F. Films were left in a fixing solution for at least 10 minutes and were washed thoroughly in cold running water before drying.

To simplify the description of the method whereby a quantitative x-ray absorption value was derived, one tooth slab, No. 22, has been selected, and the average unit absorption value for its dentin obtained. This particular slab was

roentgen rayed on films 711 A, 711 B, and 711C with other slabs of its group and the necessary penetrometers. Several points were selected in the crown and root portions of the dentin where the density appeared uniform and unaffected by tissue changes or density gradient. An effort was made to avoid distinct lines of density variation such as occurs at the edge of the slab or adjacent to a root canal. The densities for each of these points was read on all 3 films by means of the Capstaff-Purdy densitometer (11). A circle 0.75 mm. in diameter was involved in a single reading with this instrument.

Densitometer readings were also made on at least 2 points across each step of the aluminum penetrometers on every film. Average readings were then com-

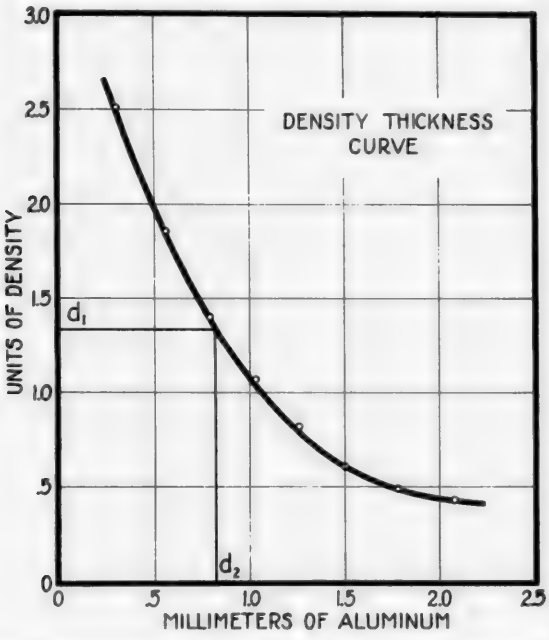

FIG. 2. Typical density-thickness curve. A densitometer reading d_1 in the dentin is interpolated into a density value in mm. aluminum d_2 as indicated.

puted for each step and were plotted against the average step thicknesses. Thus a density-thickness curve (*fig. 2*) was produced for each film, in which the ordinates represent units of density and the abscissae millimeters of aluminum. From this curve an interpolated value in millimeters of aluminum can be found which corresponds to the density of any selected point in the dentin of the tooth slab. I.V.'s[2] thus determined were made comparable slab-to-slab by dividing them by the respective slab thicknesses. Table I gives the average densitometer readings, corresponding I.V.'s and unit I.V.'s from the films of slab No. 22. The average unit I.V.'s are exemplary of values given in subsequent tables.

[2] I.V. = interpolated value in mm. aluminum.

Only 5 of the slabs contained sufficient enamel to permit roentgen absorption measurements to be made on that tissue. In these cases, the standard of comparison was a 6 per cent alloy of zinc and aluminum, the small amount of zinc being added to provide the aluminum an increased mass absorption coefficient comparable to that of normal enamel. This was in keeping with the finding that the standard for obtaining I.V.'s of a given structure must have a similar average mass coefficient of absorption (12). For the same reason, pure aluminum served as the standard for finding dentin I.V.'s. Hence, interpolated values for enamel were differentiated from those for dentin by calling them "alloy values" and were obtained from density-thickness curves for alloy penetrometers rather than

TABLE I

The average unit I.V. for dentin of tooth 22

	D.R. AVERAGE	I. V.* MM. Al	*T*, MM. THICK	$\frac{\text{I.V.}}{T}$
Film 711 A				
Crown	1.596	0.335	0.432	0.77
Root	1.613			.74
Film 711 B				
Crown	1.603	.318		.73
Root	1.626	.299		.69
Film 711 C				
Crown	1.590	.323		.74
Root	1.603	.310		.71
Average of 3 films				
Crown				.75
Root				.71
Entire				.73

* From the density-thickness curves for each film respectively.

pure aluminum ones. Like I.V.'s, alloy values were divided by slab thicknesses for purposes of comparison.

RESULTS

The unit A.V.'s[2] and I.V.'s for enamel and the coronal root, and entire dentin of all the teeth are indicated in Table II. The average unit absorption value for enamel is 0.97 mm. alloy, a figure only 3 per cent lower than unity, the expected unit value for normal enamel; and for all the dentin is 0.74 mm. Al, a figure far lower than 0.94, the unit value reported by Van Huysen for normal dentin.

From 7 slabs containing both coronal and root dentin, it is found that the latter absorbs on the average 4.7 per cent less radiation than the former. Normal teeth, on the other hand, reveal a gross variation between coronal and root dentin of approximately 6 per cent (6). If the teeth are arranged in

[2] A.V. = alloy value in mm. 6 per cent Zn-Al alloy.

the order of their root dentin I.V.'s and the condition or absence of their crowns noted (Table II) some interesting facts are uncovered: 1. the 2 unerupted and partially erupted teeth have I.V.'s above average, 2. comparatively sound erupted teeth have unit I.V.'s below average, and 3. teeth whose crowns are missing or otherwise show the effects of extreme attrition or caries, tend to have higher unit I.V.'s than comparatively sound erupted ones. An example of this tendency is found in a comparison of the upper first incisors. The right first incisor, No. 8, was a comparatively sound tooth with only the incisal enamel

TABLE II

Average roentgen-ray absorption of hereditary opalescent teeth #

TOOTH		CORONAL CONDITION	SLAB THICKNESS	UNIT A.V. ENAMEL	UNIT I.V. DENTIN		
No.	Name				Crown	oot	Entire
9	U L 1st In.	Missing	0.306	—	—	0.79	—
2	U R 2nd Mo.	Unerupted	.504	0.97	0.80	.77	0.79
20	L L 2nd Prem.	Missing	.524	—	—	.77	—
10	U L 2nd In.	Missing	.404	—	—	.76	—
13	U L 2nd Prem.	Extreme caries	.601	—	—	.76	—
31	L R 2nd Mo.	Extreme attrition	.426	—	—	.76	—
15	U L 2nd Mo.	Sound, partially erupted	.361	—	.77	.73	.75
27	L R Canine	Missing	.572	—	—	.72	—
22	L L Canine	Cusp tip worn	.432	.99	.75	.71	.73
21	L L 1st Prem.	Missing	.560	—	—	.70	—
12	U L 1st Prem.	Sound	.564	.97	.73	.70	.71
8	U R 1st In.	Incisal enamel worn	.579	—	.74	.70	.72
7	U R 2nd In.	Enamel fractured off	.595	—	.73	.69	.72
4	U R 2nd Prem.	Sound	.544	.97	.70	.67	.69
24	L L 1st In.	Incisal enamel worn	.537	.94	.67	—	—
Average			.500	.97	.74	.73	.74
Maximum			.601	.99	.80	.79	.79
Minimum			.306	.94	.67	.67	.69
Standard deviation				.01	.04	.03	.03
Range of variation				.05	.13	.12	.10
Number of teeth examined*				5	8	14	7

Arranged in descending order of root dentin unit I.V.'s.
* Total = 15.

destroyed by attrition, whereas the left No. 9, was extremely affected, the whole crown being destroyed. The root dentin unit I.V. of No. 8 is 0.70 mm. Al; that of No. 9 is 0.79 mm. Al.

Still another tendency in the distribution of unit I.V.'s is that of the anterior teeth to fall in the category of those below average, while the posteriors fall into that of above average unit I.V.'s. This appears logical in the light of the functional characteristics of anterior and posterior dentitions. No significant differences between the average unit I.V.'s for root dentin of anterior and posterior teeth, nor of maxillary and mandibular teeth, could be found.

Calculation of a theoretical Unit I.V.: Since variations in the roentgen-ray absorption values of dental tissues are traceable to variations in their chemical compositions, especially their inorganic content (12), it is of interest to compare analyses of normal and opalescent teeth together with their experimental unit x-ray absorption values. This is done in Table III. Given these figures, it is

TABLE III

X-ray absorption and contents of normal and opalescent teeth

ANALYSIS OF TEETH BY HODGE, FINN, ROBINSON, MANLY, MANLY, VAN HUYSEN AND BALE. (13)	ENAMEL			DENTIN		
	Normal	Opalescent teeth	Difference	Normal	Opalescent teeth	Difference
			per cent			*per cent*
X-ray absorption value	1.00*	0.97	−3	0.94**	0.74	−22
Water content, per cent whole	2.3	2.7	—	13.2	15.5	+17
Organic content, per cent whole	4.3	1.7	—	26.6	31.0	+16
Inorganic content, per cent whole	93.4	95.6	+2	60.2	53.5	−11

* Hodge, Van Huysen, Warren (9).
** Van Huysen (6).

TABLE IV

Calculations of mass absorption coefficients for normal and hereditary opalescent dentin

ELEMENT	PER CENT BY WEIGHT		μ_m AT 0.7 Å		CONTRIBUTION TO μ_n	ELEMENT	HER CENT BY WEIGHT		μ_m AT 0.7 Å		CONTRIBUTION TO μ_o
Ca	22.9	×	18.60	=	425.9	Ca	19.0	×	18.60	=	353.4
P	11.1	×	8.00	=	88.1	P	8.8	×	8.00	=	70.4
H_2O	31.5	×	2.09	=	65.8	H_2O	36.7	×	2.09	=	76.7
C	10.5	×	.60	=	6.3	C	15.5	×	.60	=	9.3
N	3.6	×	.87	=	3.1	N	4.9	×	.87	=	4.2
O	19.6	×	1.22	=	23.9	O	11.1	×	1.22	=	13.5
Total normal dentin = 613.1 = μ_n						Total opalescent dentin = 527.5 = μ_o					

possible to compute a theoretical unit I.V. for opalescent dentin for comparison with the experimental value.

From the classical equation for roentgen ray absorption

$$I = I_o e^{-\mu x}$$

it may be shown that

$$\mu_{Al} \, (\text{I.V.}/x)_d = \mu_d$$

in which μ_{Al} and μ_d = the mass absorption coefficients of aluminum and dentin respectively, and $(\text{I.V.}/x)_d$ = the unit I.V. for that tissue. Then

$$\frac{(\text{I.V.}/x)_o}{(\text{I.V.}/x)_n} = \frac{\mu_o}{\mu_n}$$

where the subscript o refers to opalescent dentin, and n to normal dentin.

Calculations of μ_o and μ_n at 0.7 Å, probably the predominant wave length of x-rays produced at 30 kv., are made in table IV. Substituting these values and the unit I.V. for normal dentin, 0.94 mm. Al, in the above equation, gives for the unit absorption value of opalescent dentin, 0.81 mm. Al. This value is less than 10 per cent greater than the experimental average unit I.V. and is, therefore, comparable with it.

SUMMARY

1. The average experimental roentgen ray absorption value for hereditary opalescent dentin (odontogenesis imperfecta) from 15 teeth is approximately 0.74 mm. Al equivalent per mm. thickness.

2. This value compares with a calculated unit I.V. (interpolated value) based on analyses of normal and opalescent dentin and on the assumption that normal dentin absorbs x-radiation in an amount equivalent to 0.94 mm. Al per mm. thickness.

3. Coronal opalescent dentin consistently absorbs more roentgen radiation than root dentin, and in this respect is similar to normal dentin.

4. It would appear that attrition has an effect on x-ray absorption by opalescent dentin, generally causing it to be increased.

5. No significant differences are found in the average unit interpolated values for anterior and posterior opalescent teeth, nor for maxillary and mandibular ones.

6. The enamel of hereditary opalescent teeth is probably normal.

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